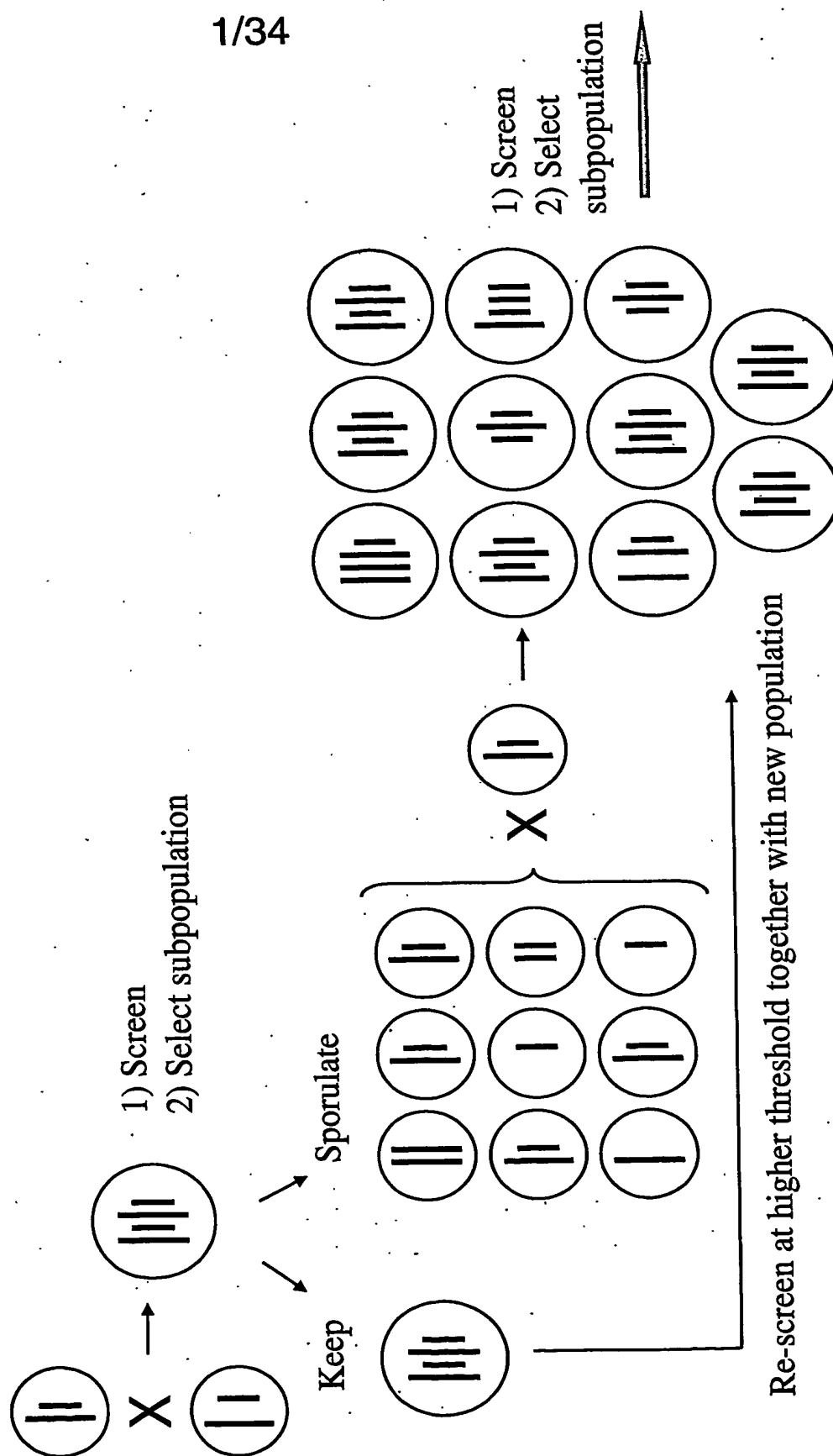


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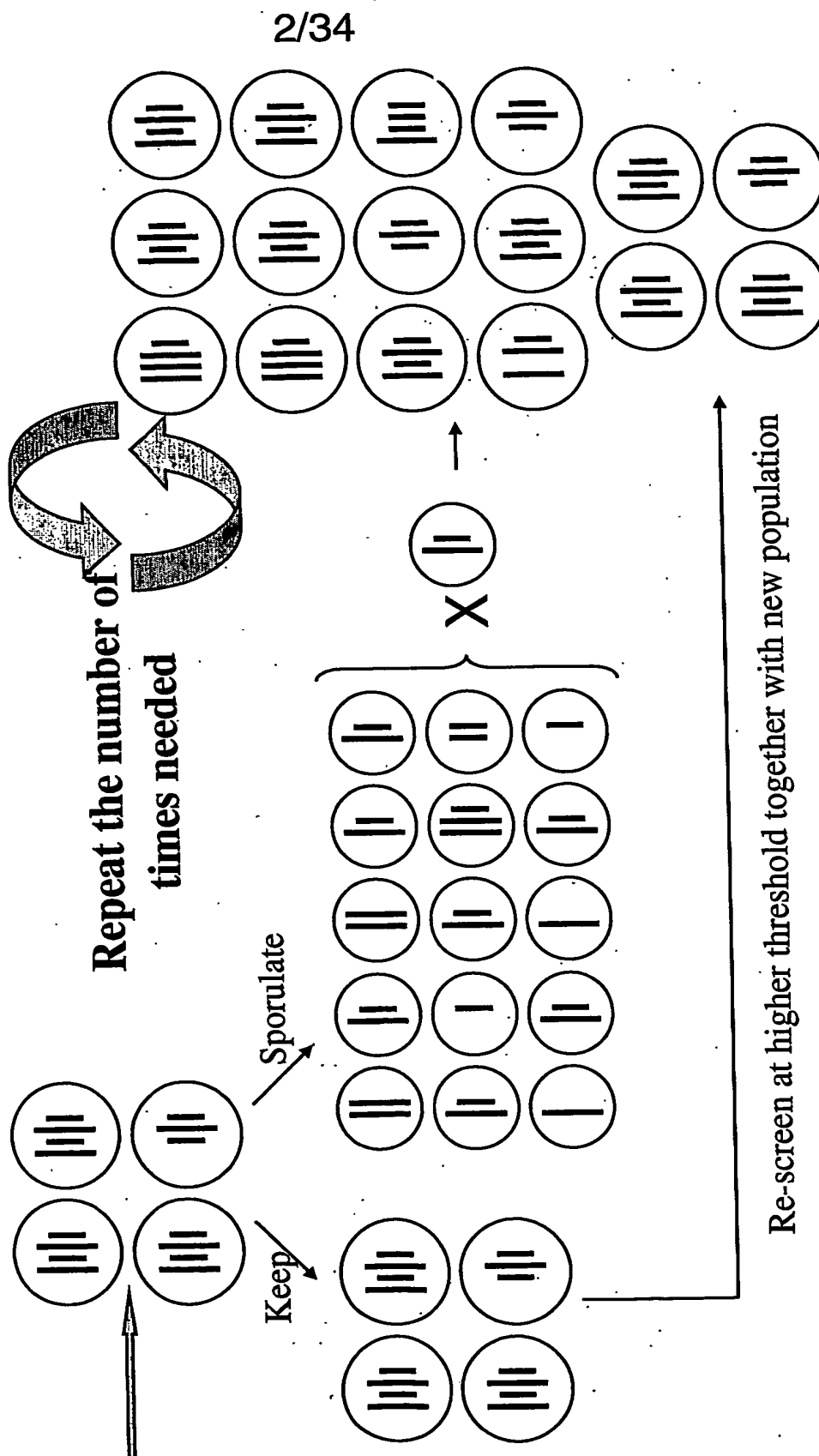
Remixing Strategy

Fig. 1



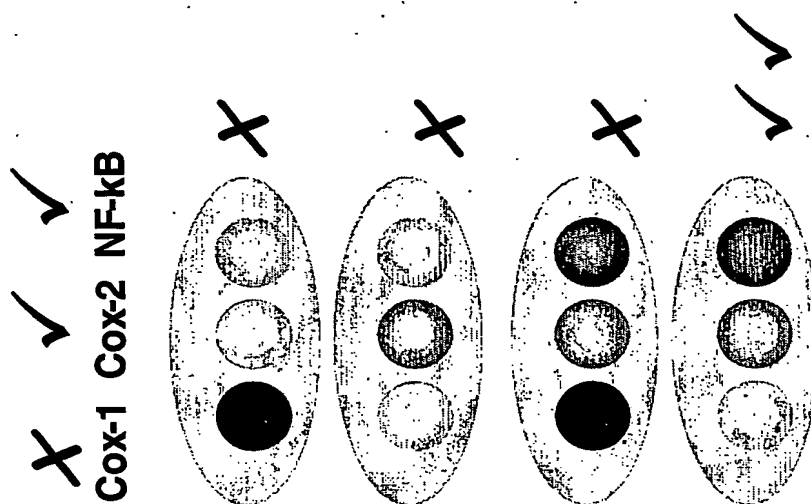
Remixing Strategy

Fig. 1 cont'd



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- Later screening rounds:
select cells that fit all the
criteria



- Initial screening rounds:
select cells that fit some
of the criteria

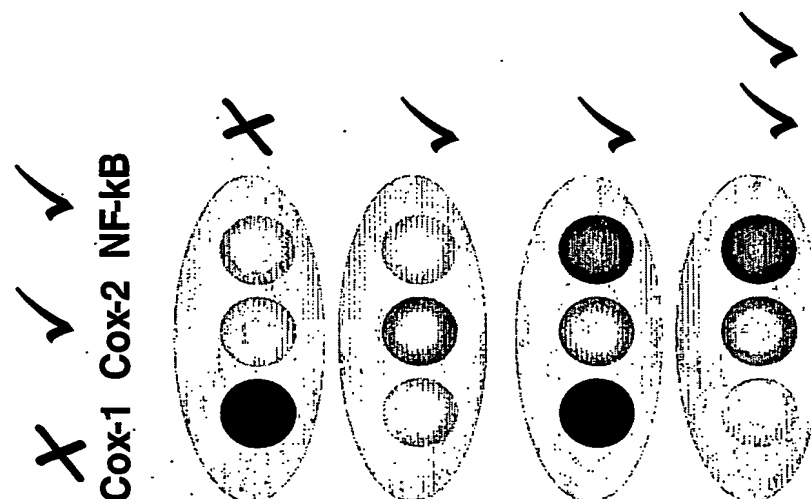


Fig. 2

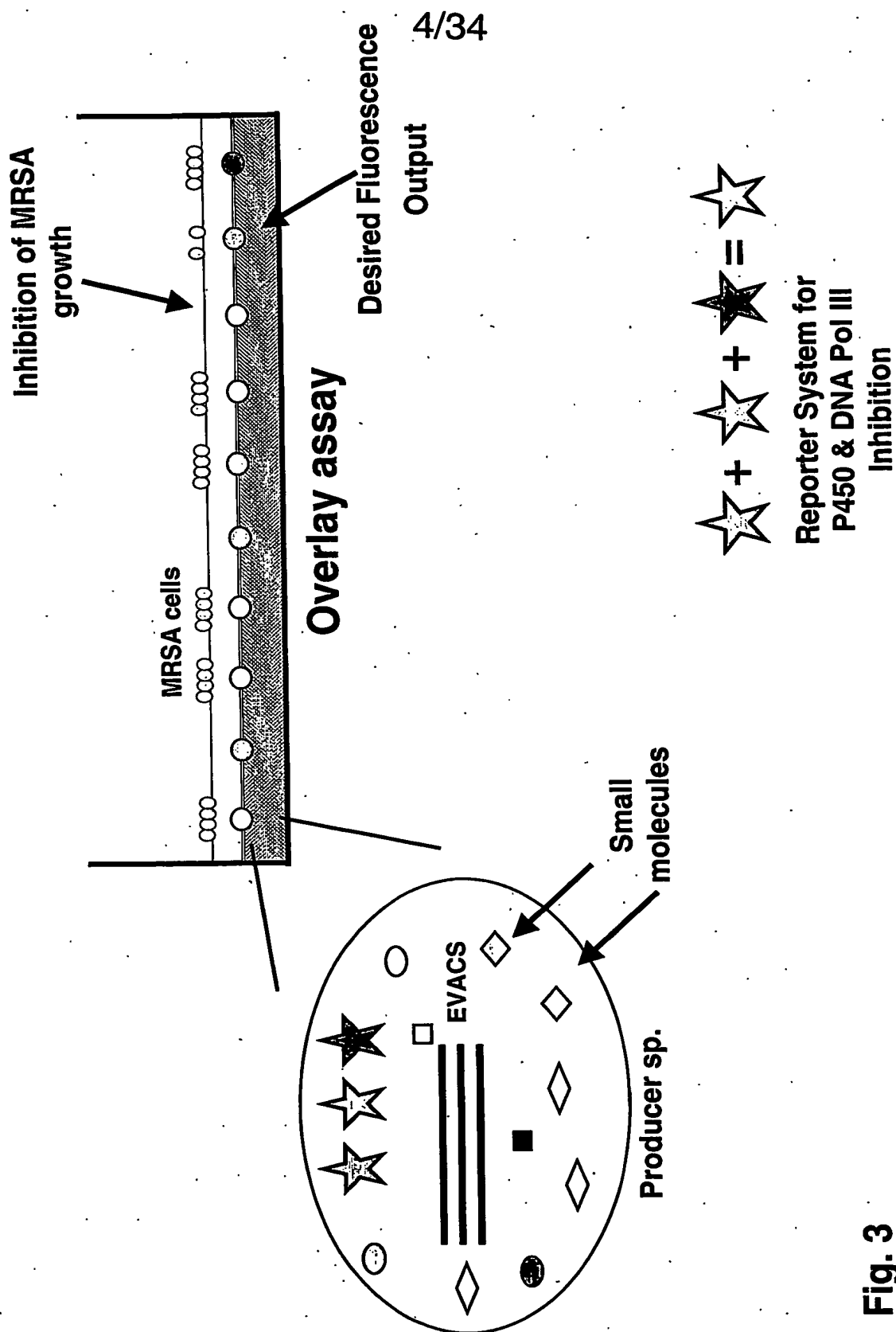


Fig. 3

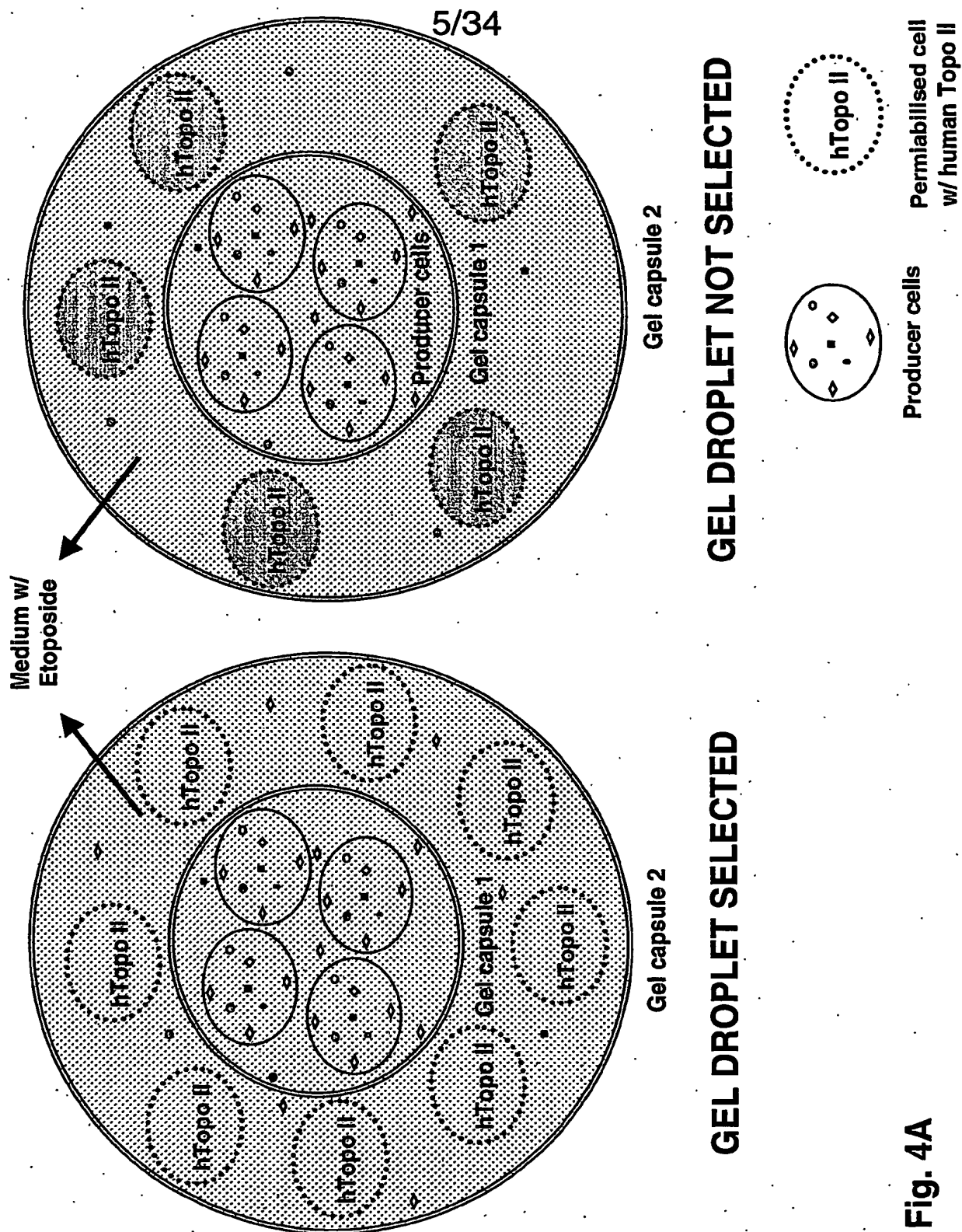


Fig. 4A

Fig. 4B

Evolving Topo II Compounds

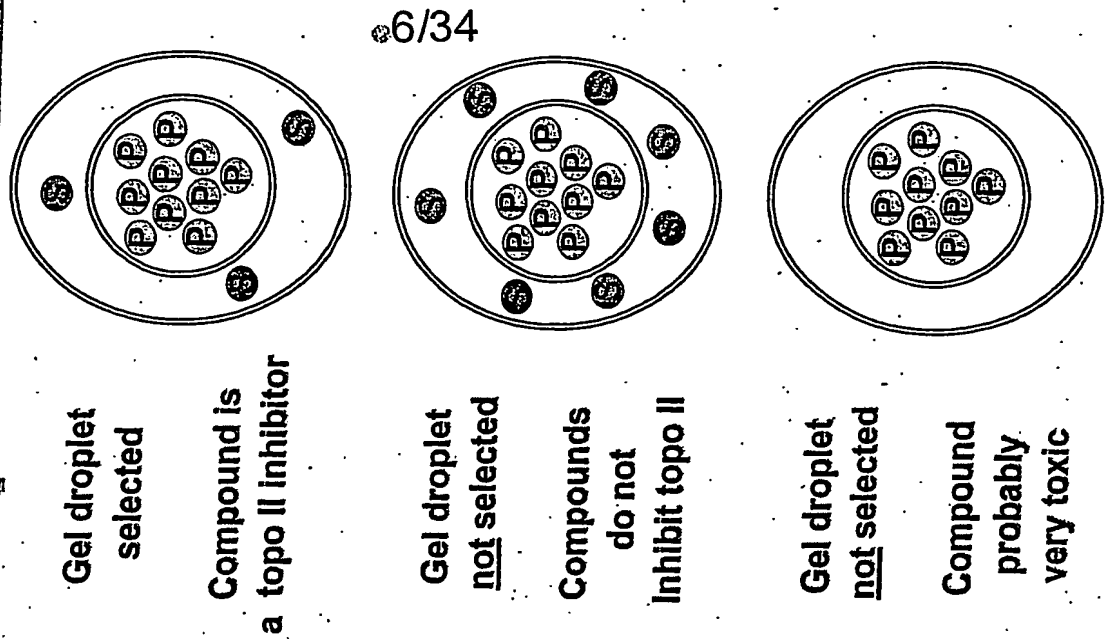
- ◆ Gel encapsulate single producer yeast
 - ◆ Allow growth to 100 cell clonal colony
 - ◆ Induce

- ◆ Gel encapsulate reporter yeast

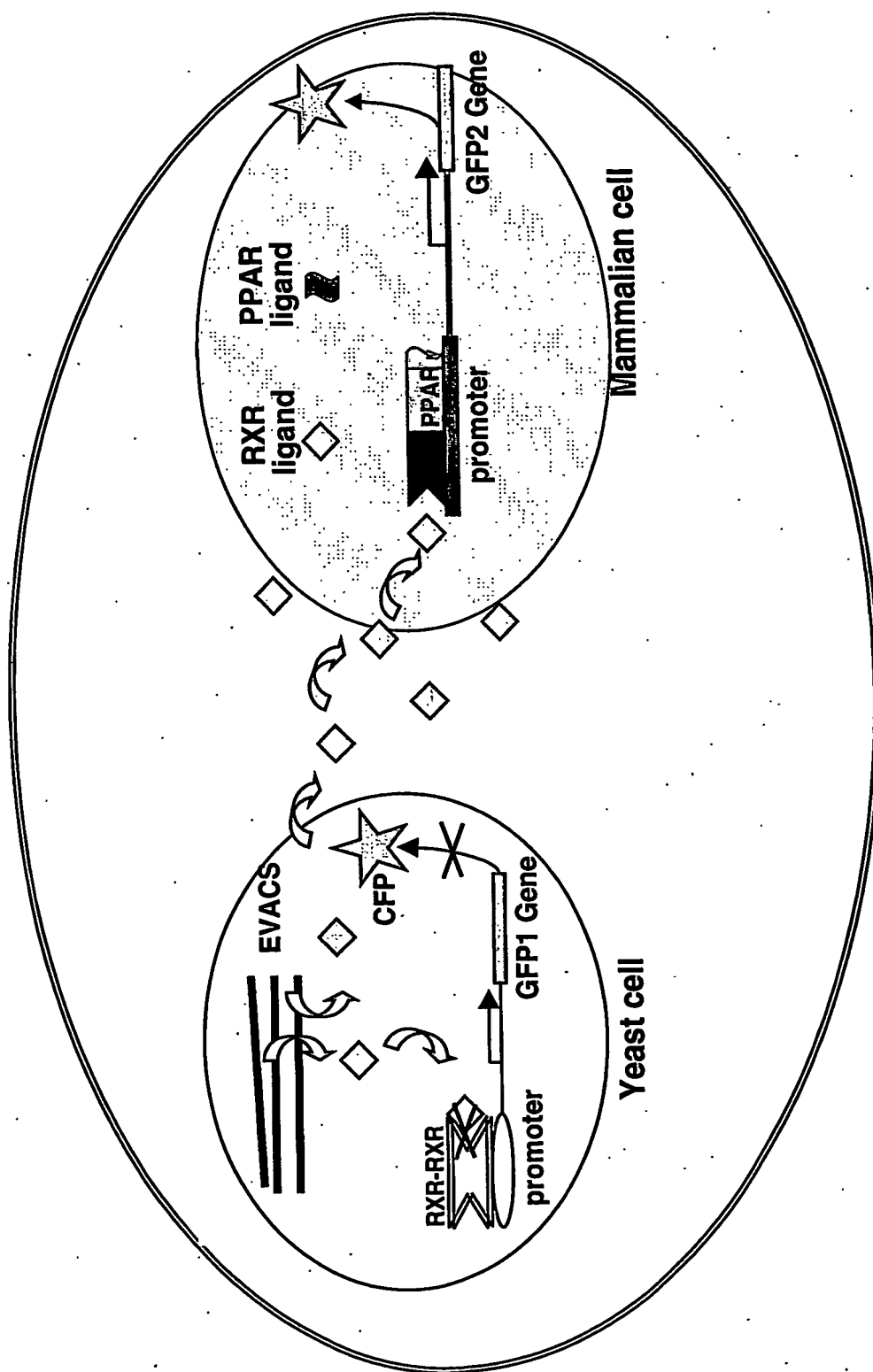
- ◆ Procedure avoids excessive reporter yeast proliferation

- ◆ Add precursor

- ◆ Screen & sort: ● (JN394) ● (JN394top2-5)



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Example of droplet selected:
No activation of RXR-RXR
and activation of RXR-PPAR γ

Gel capsule

Fig. 5

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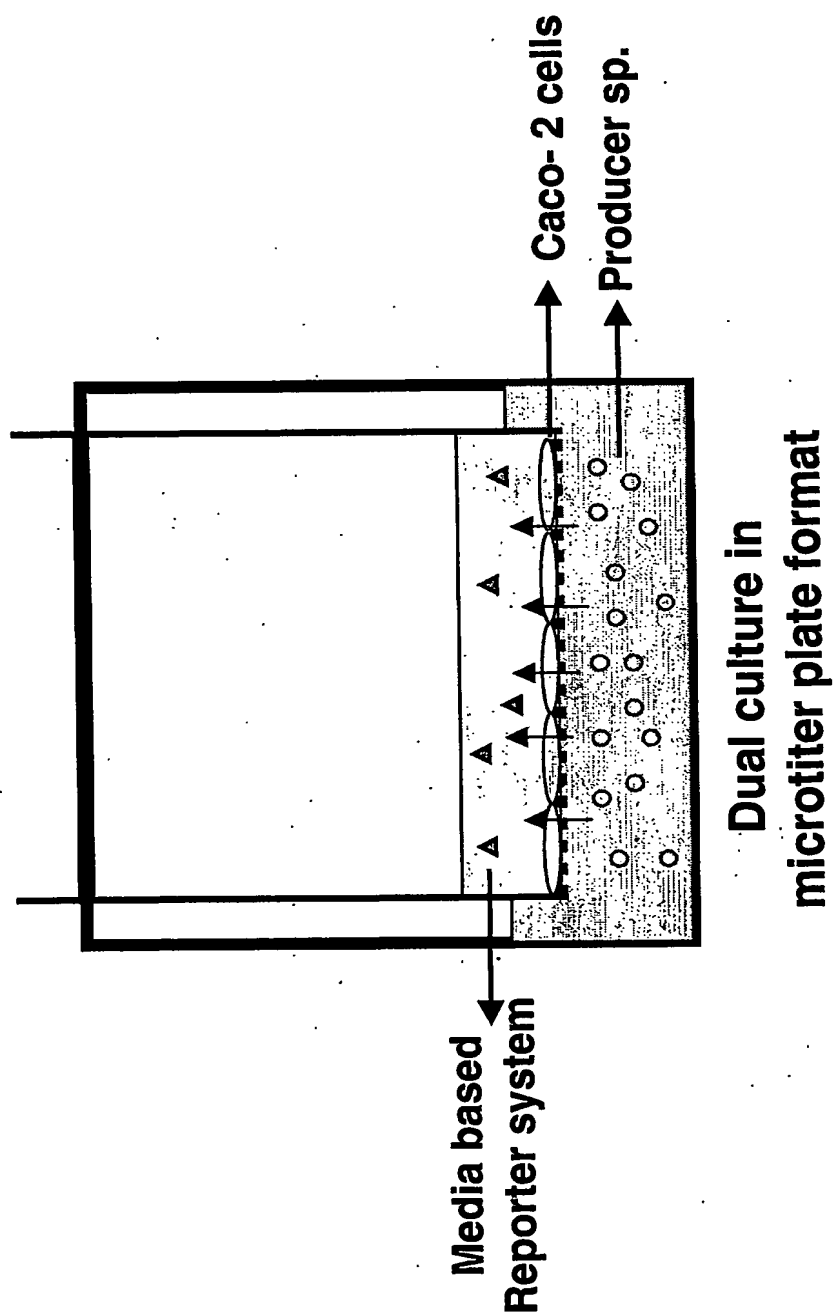


Fig. 6

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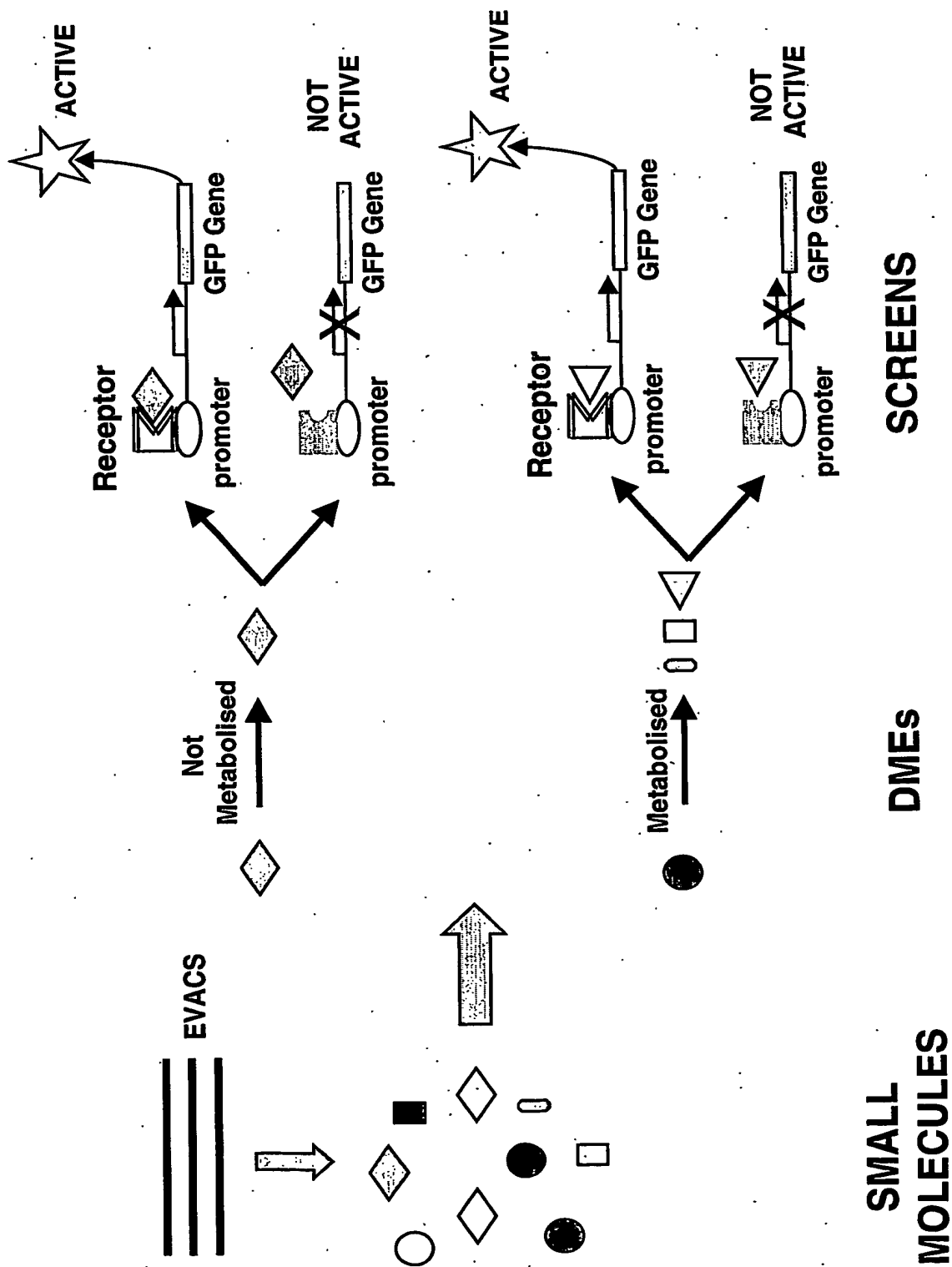


Fig. 7

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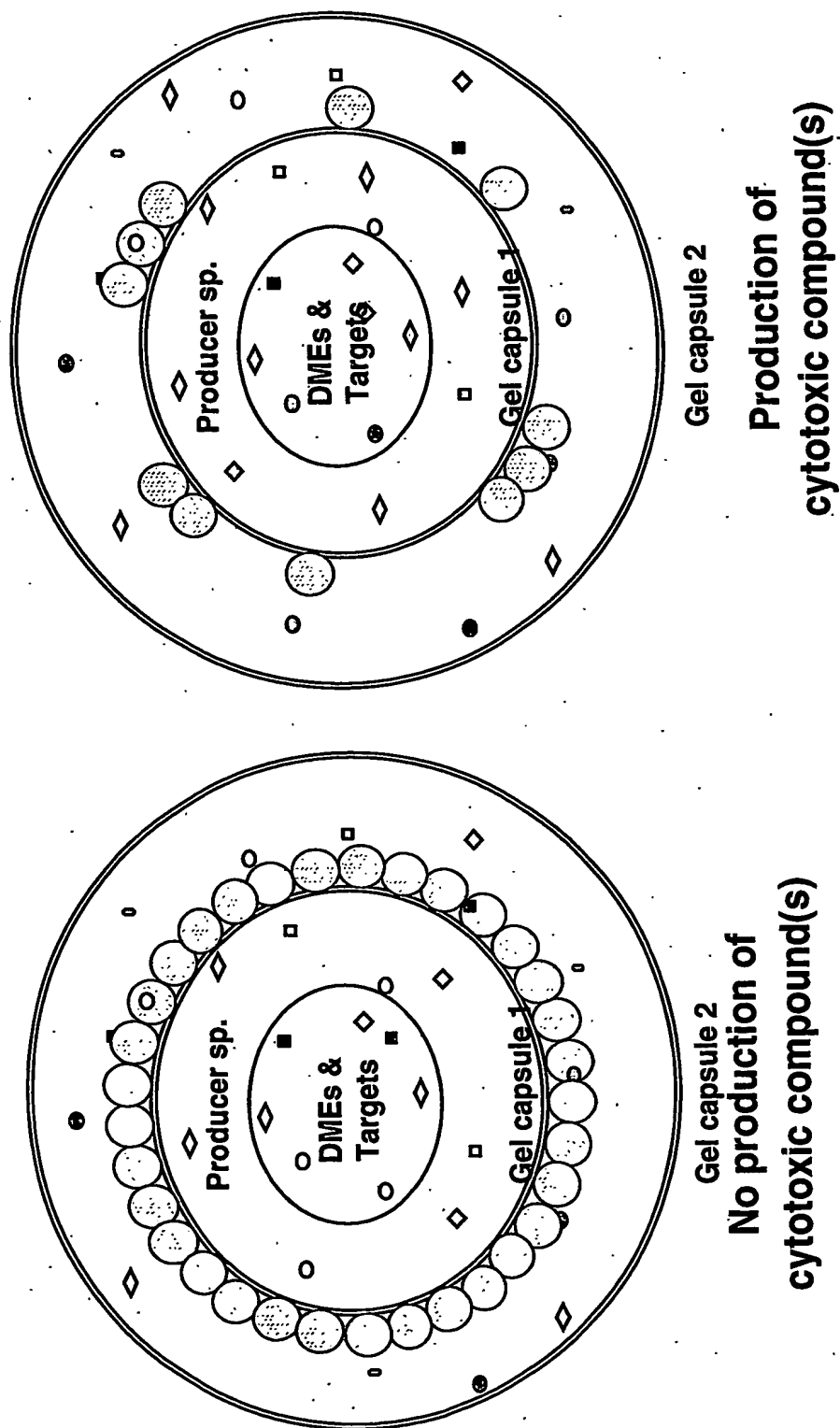
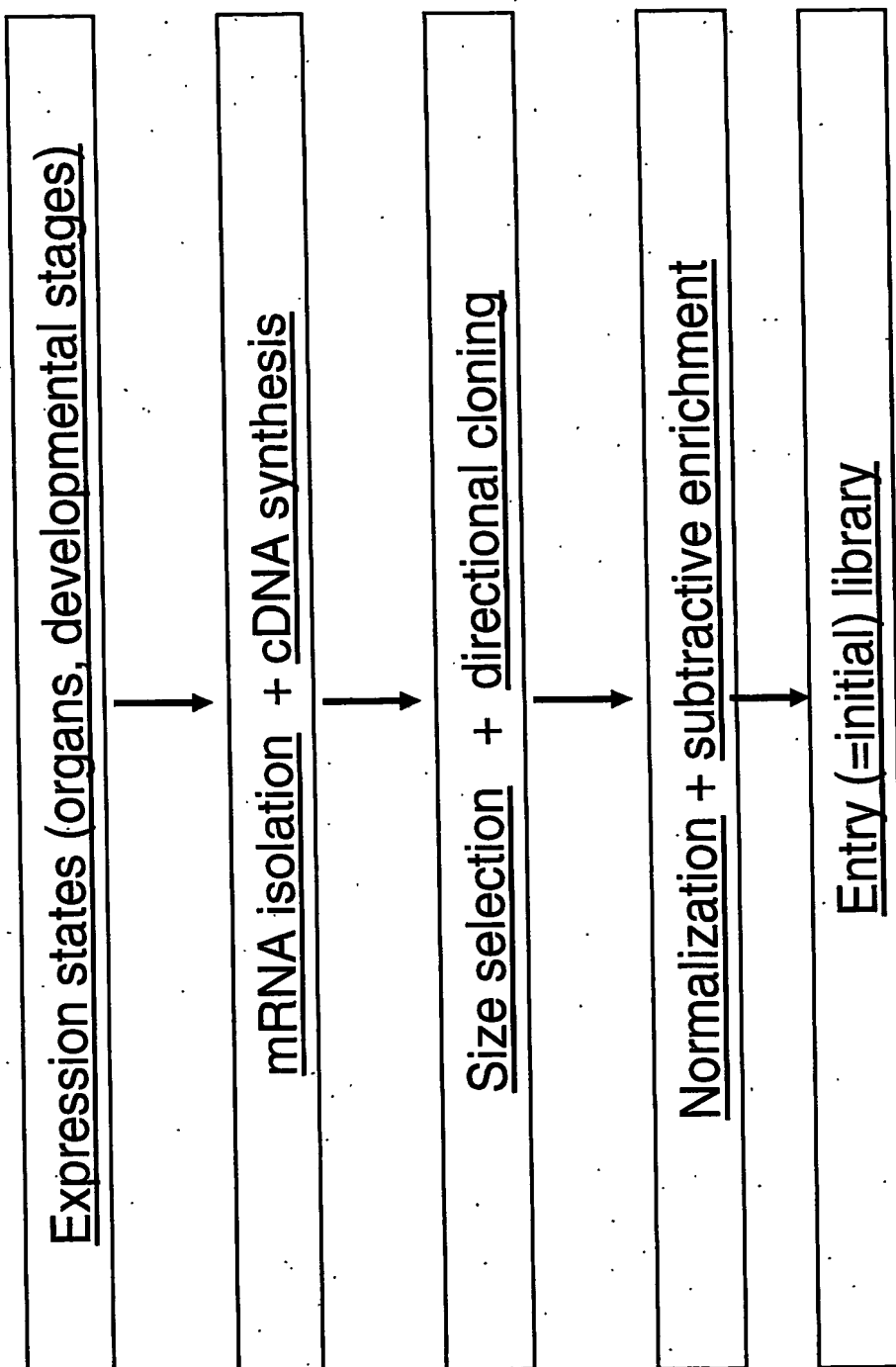


Fig. 8

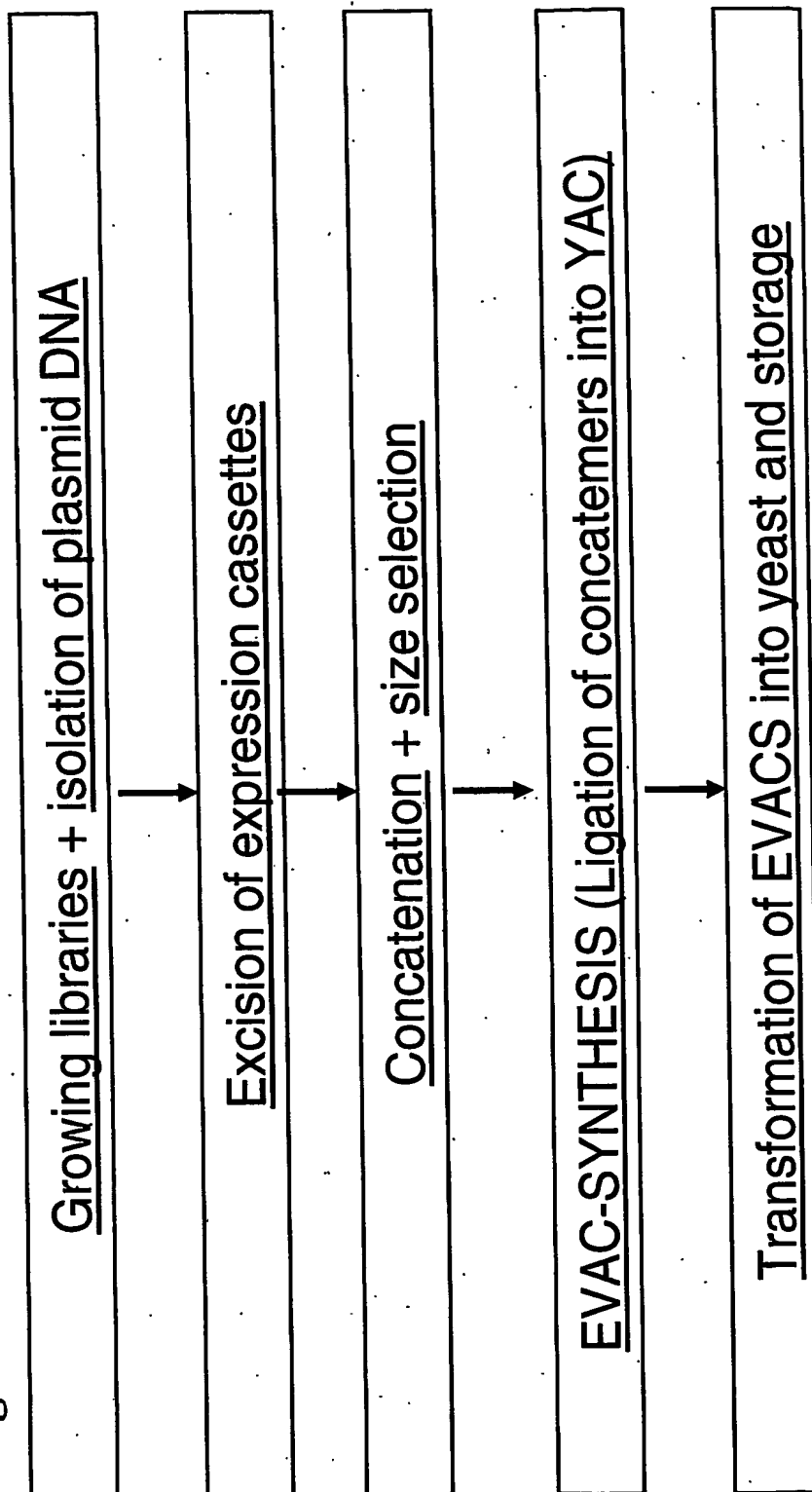
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Constructing entry libraries

Fig. 9

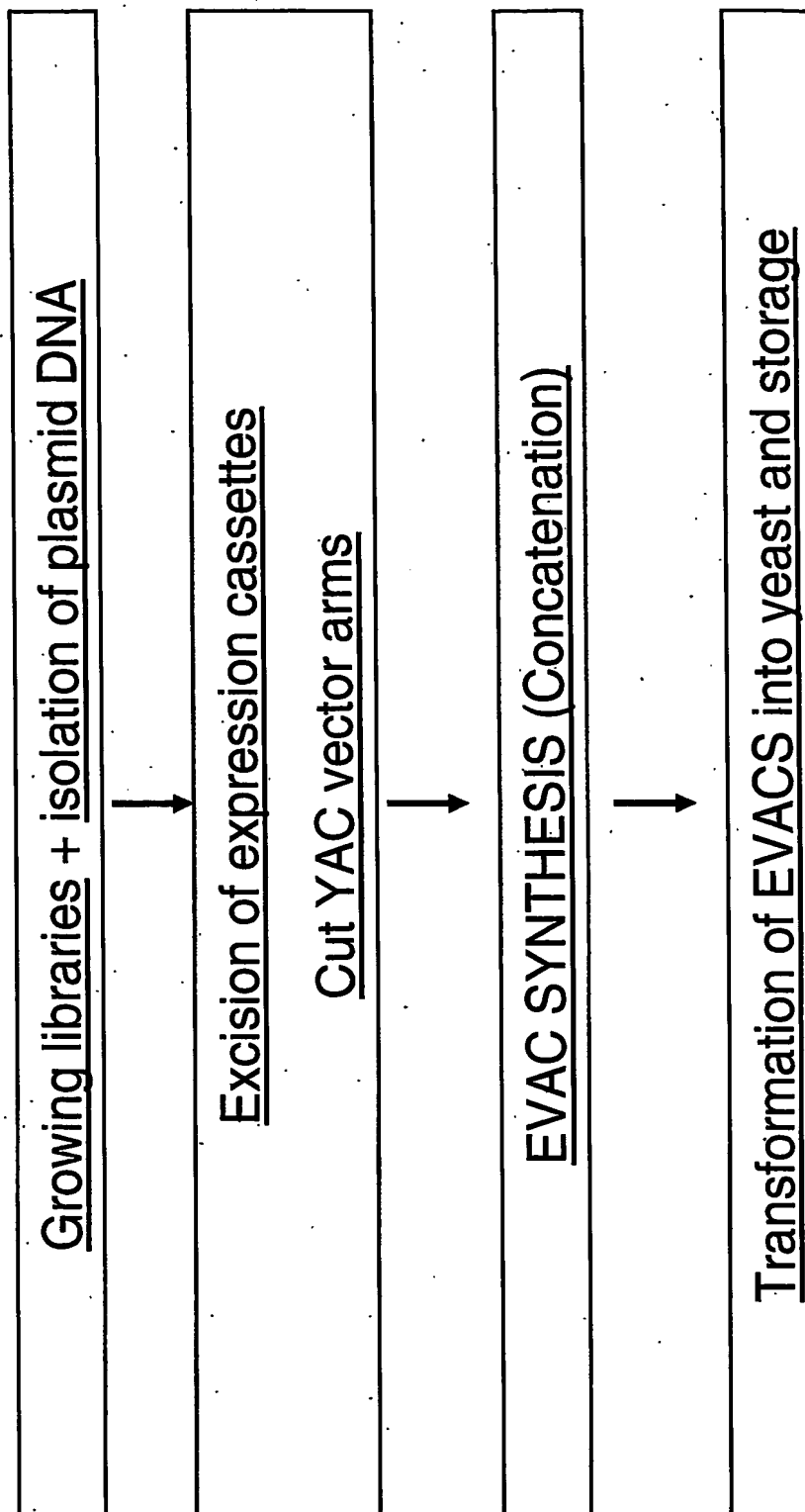


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Entry library to evolvable cell**Fig. 10a**

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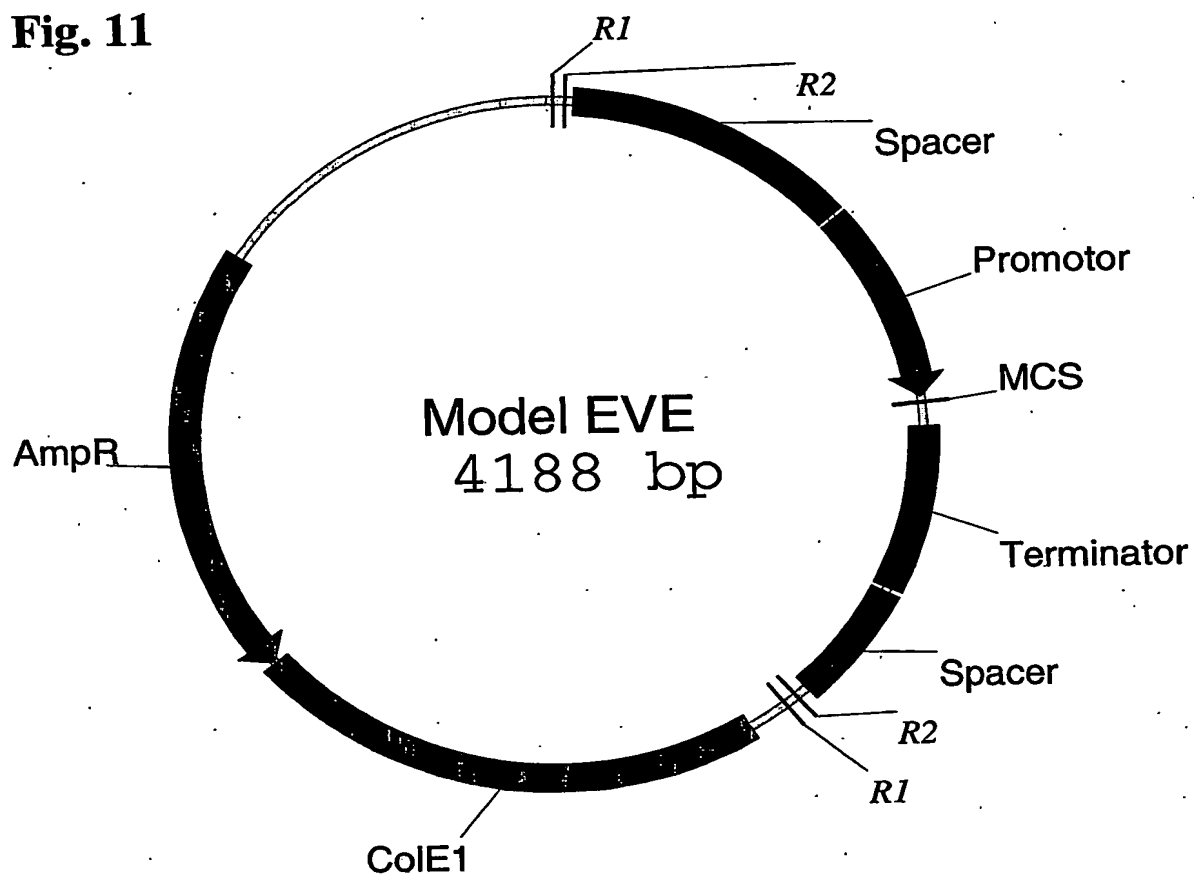
Fig. 10b **Entry library to evolvable cell**



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Model Entry Vector

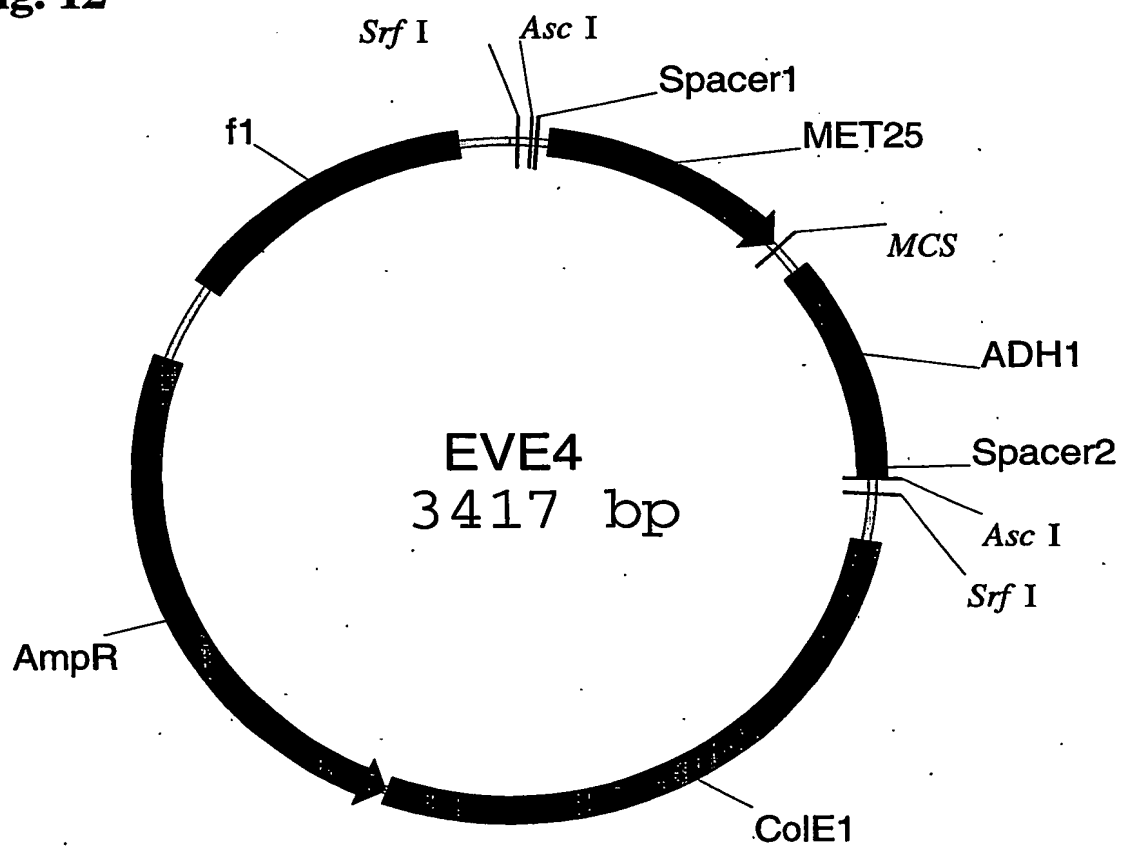
Fig. 11



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EVE4 entry vector

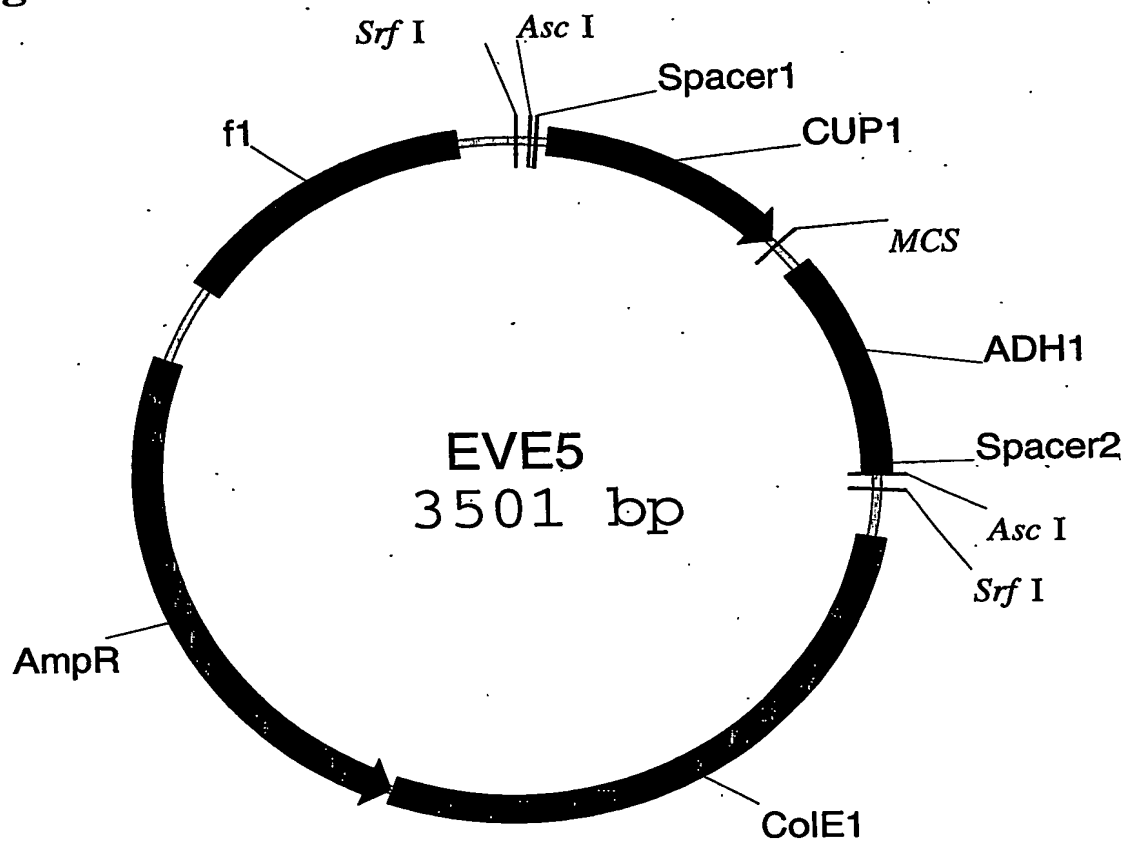
Fig. 12



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EVE5 entry vector

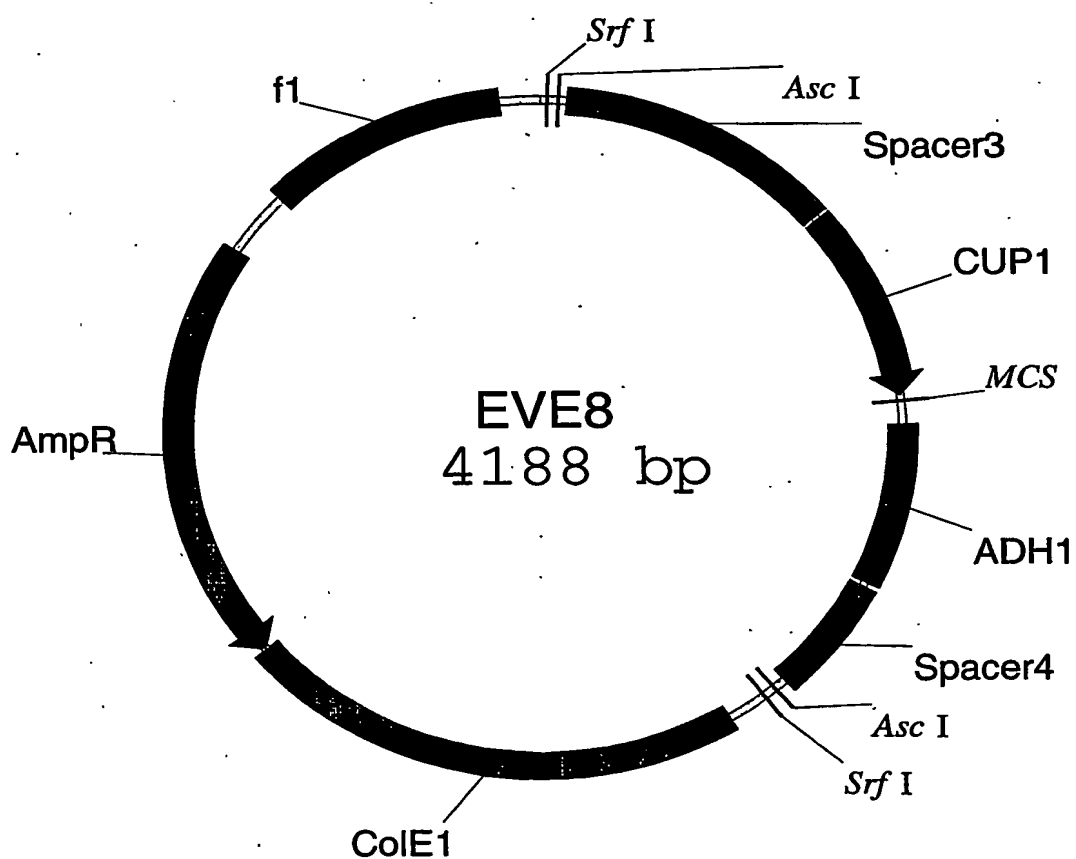
Fig. 13



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EVE8 entry vector

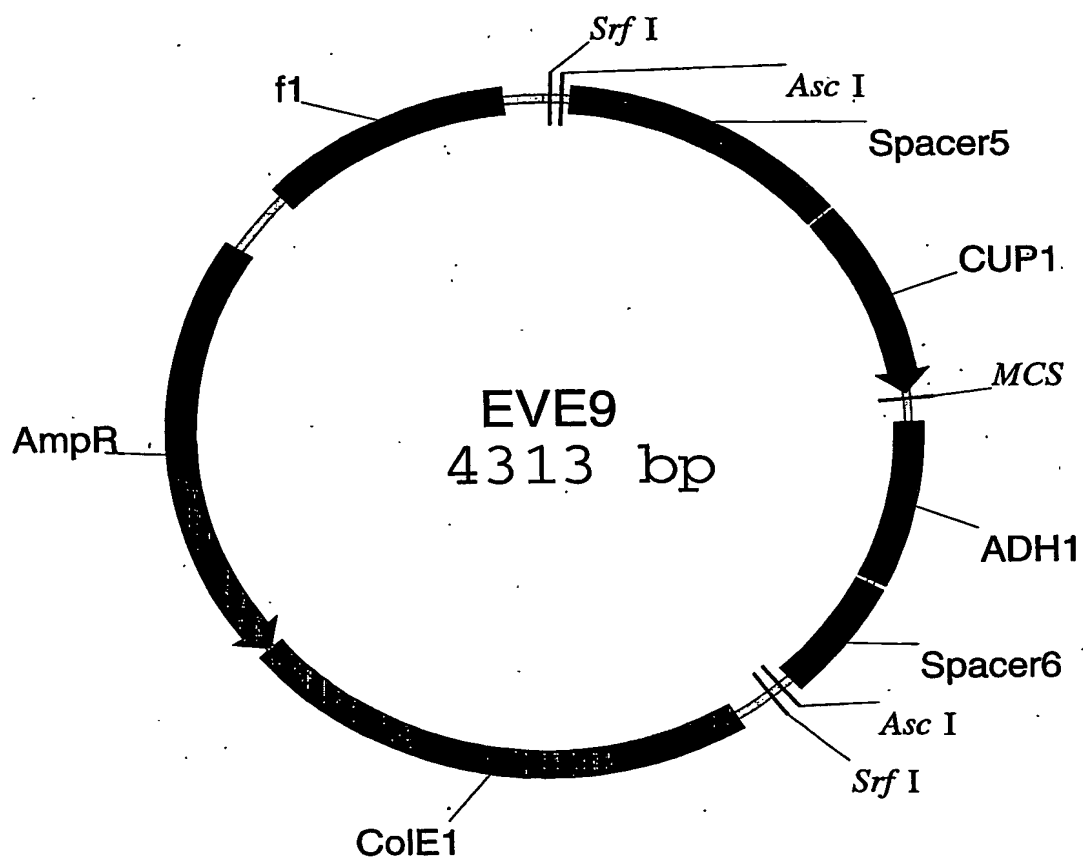
Fig. 14



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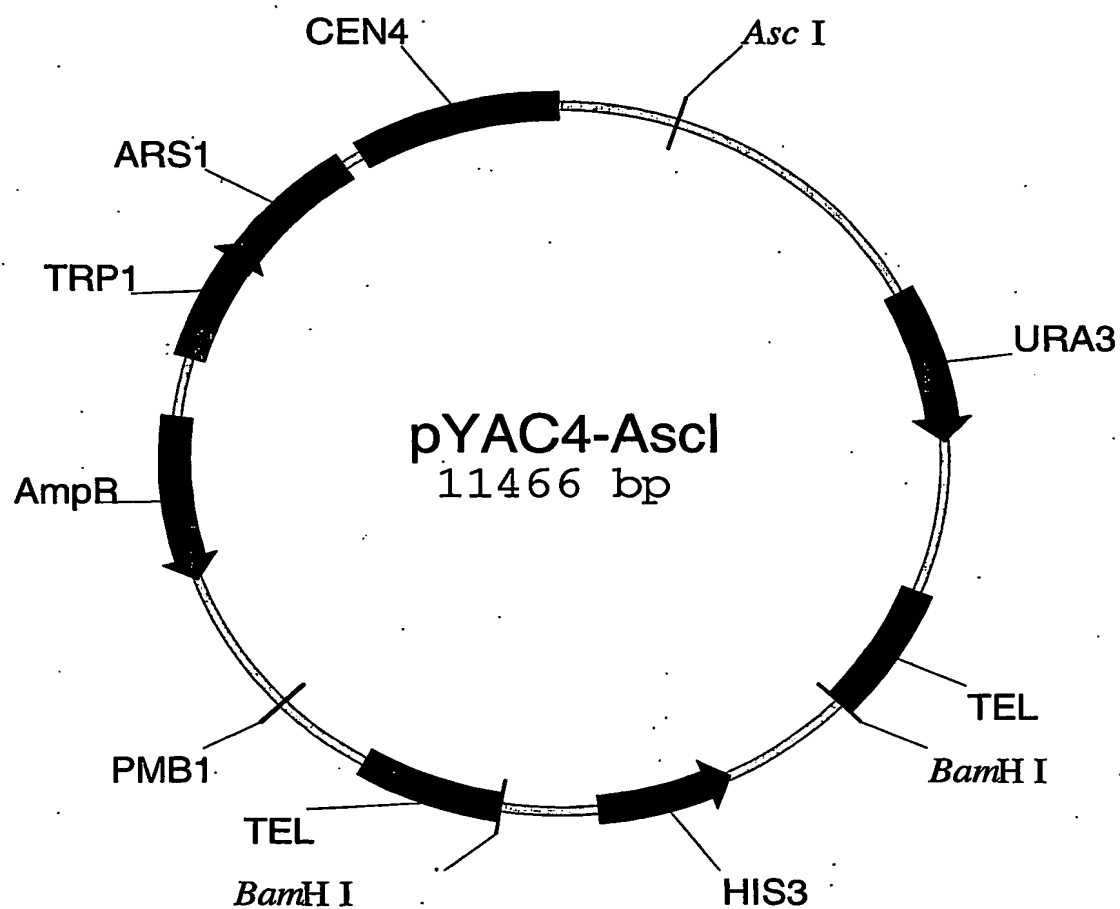
EVE9 entry vector

Fig. 15



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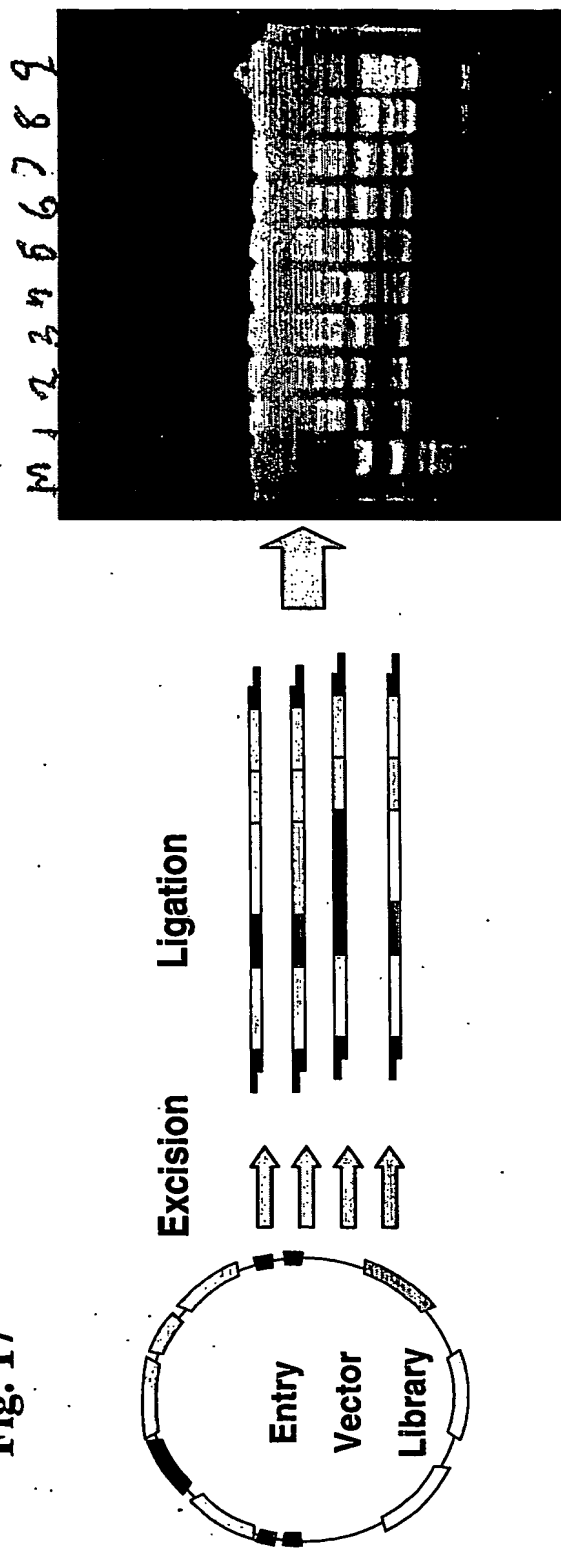
pYAC4-AscI
Vector for providing EVACS arms

Fig. 16

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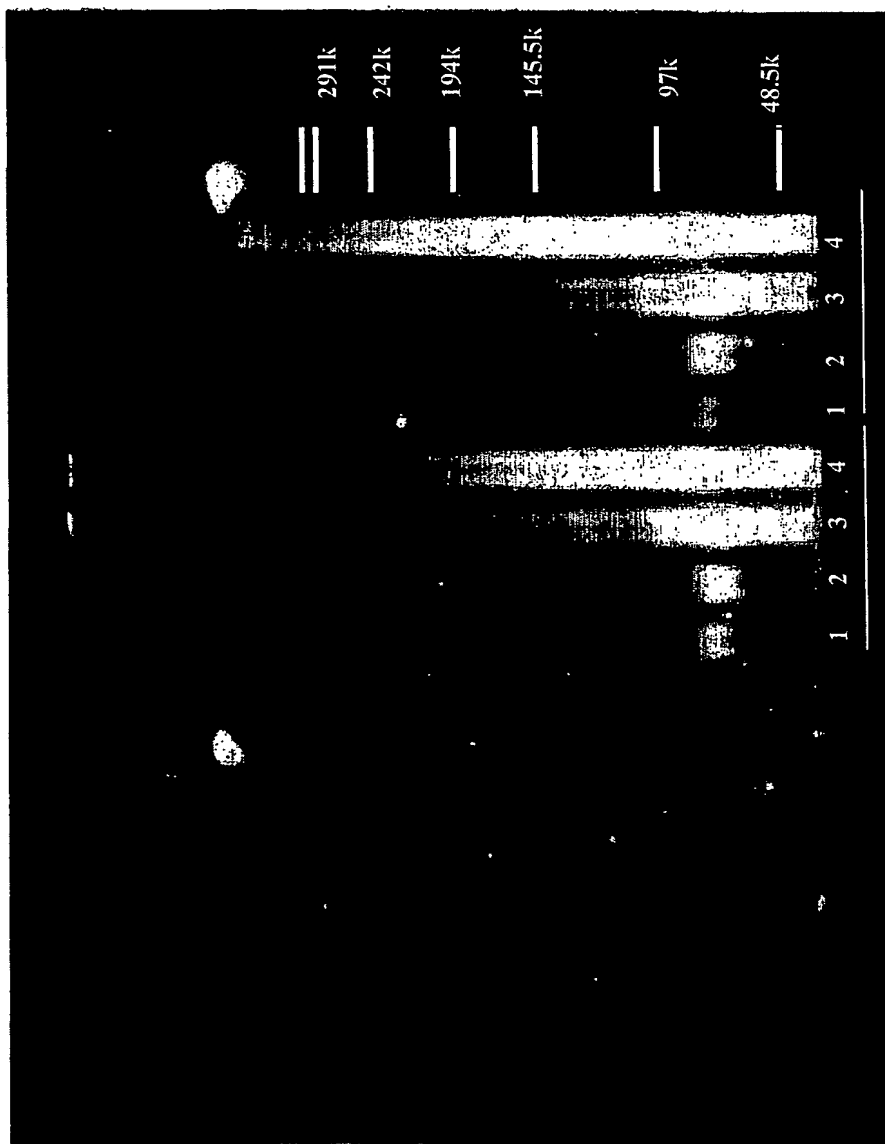
Synthesis of Concatemers

Fig. 17



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Fig. 18a



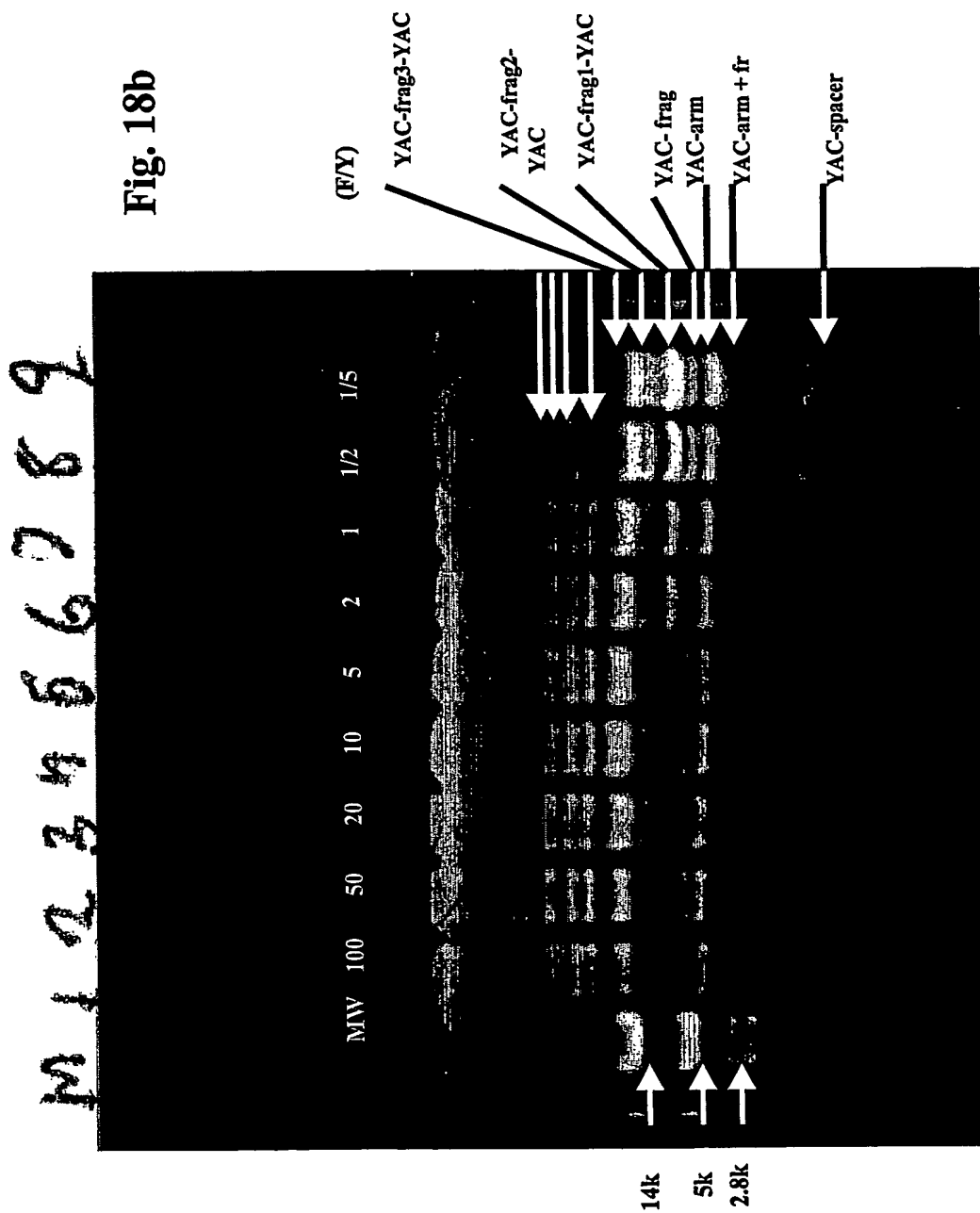
A: F/Y = 100, B: F/Y = 1000

1: fragment conc.= 1
2: fragment conc.= 2
3: fragment conc.= 5
4: fragment conc.= 10

3+4: same amount loaded on gel
But concentration in 4 = 2x
concentration in 3

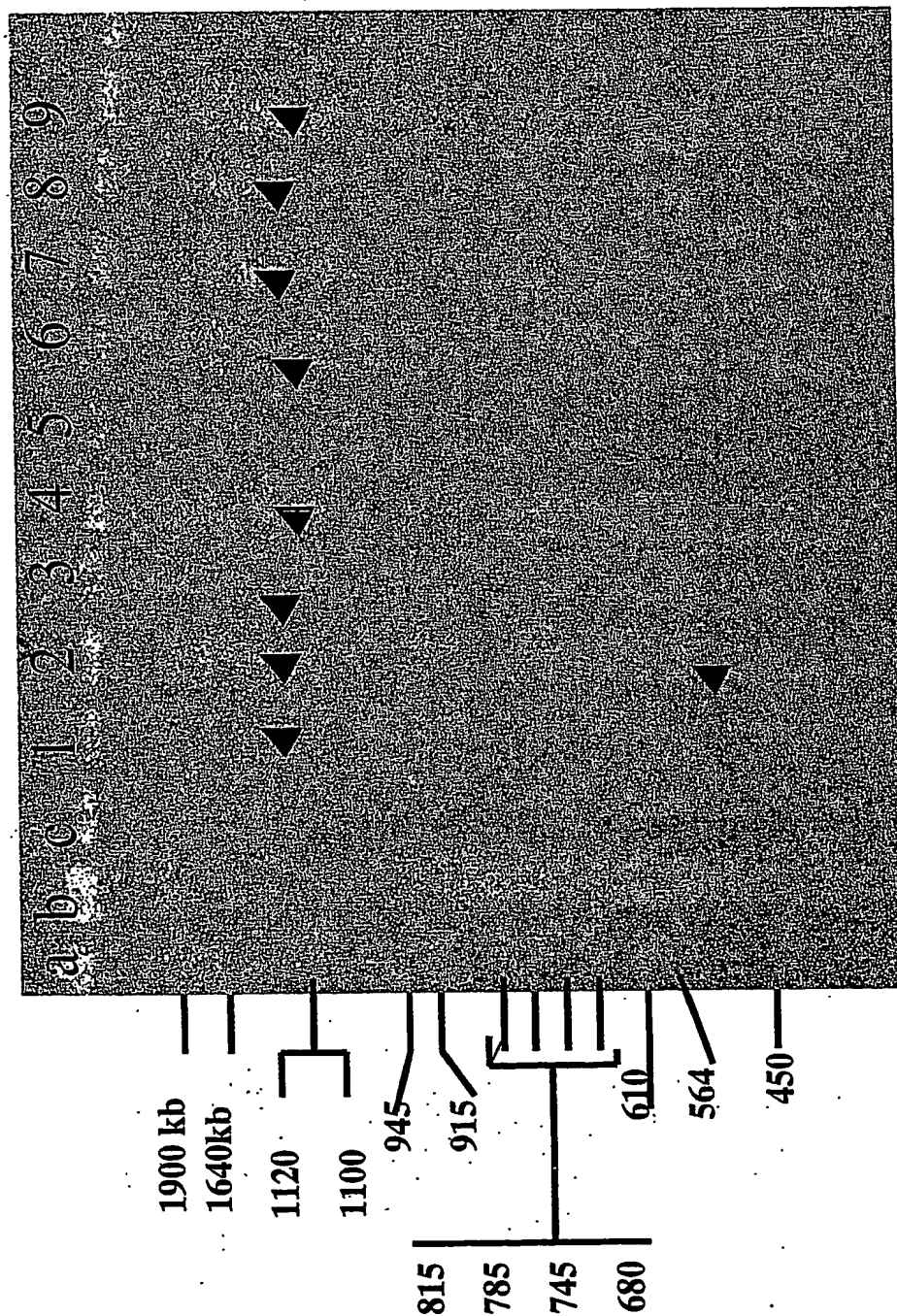
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Fig. 18b



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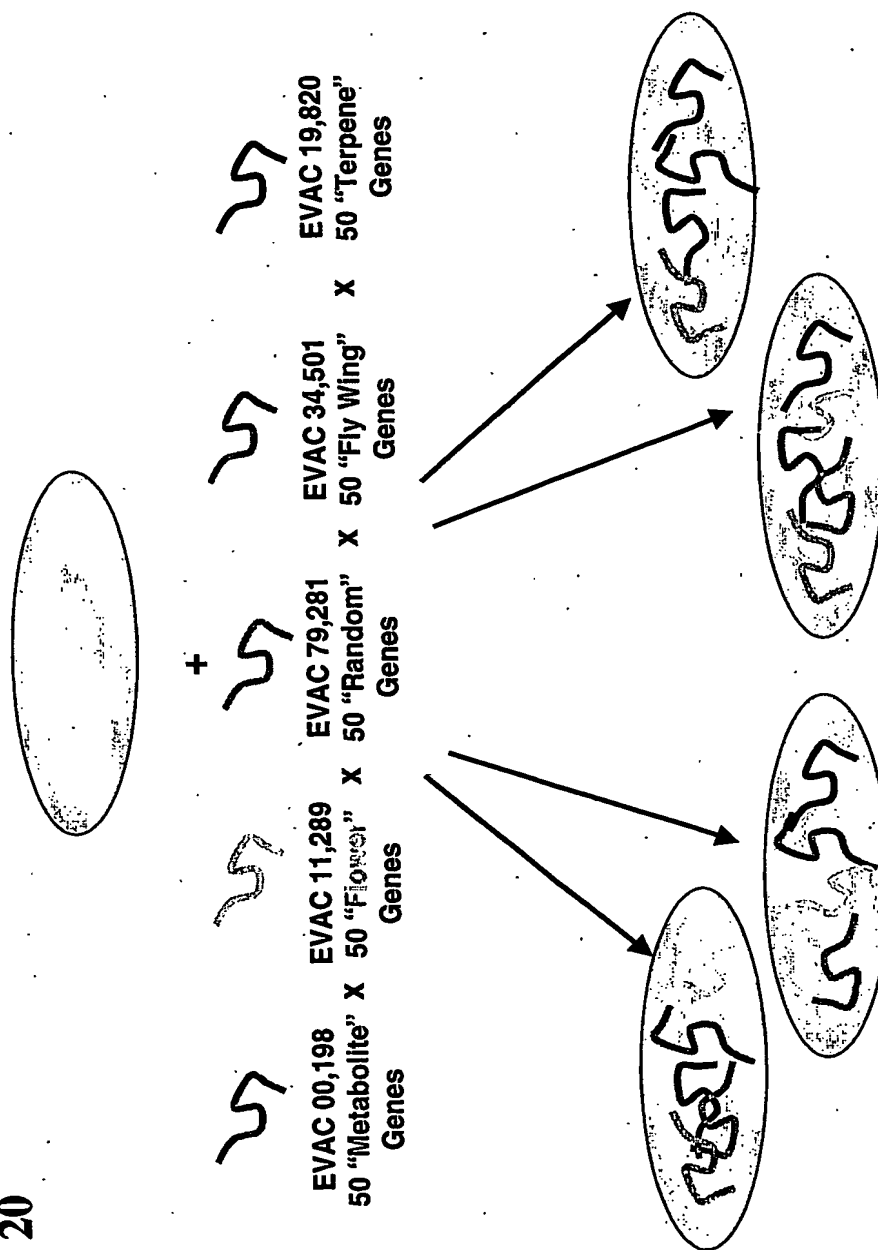
Fig. 19



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Generation of EVAC containing cell populations

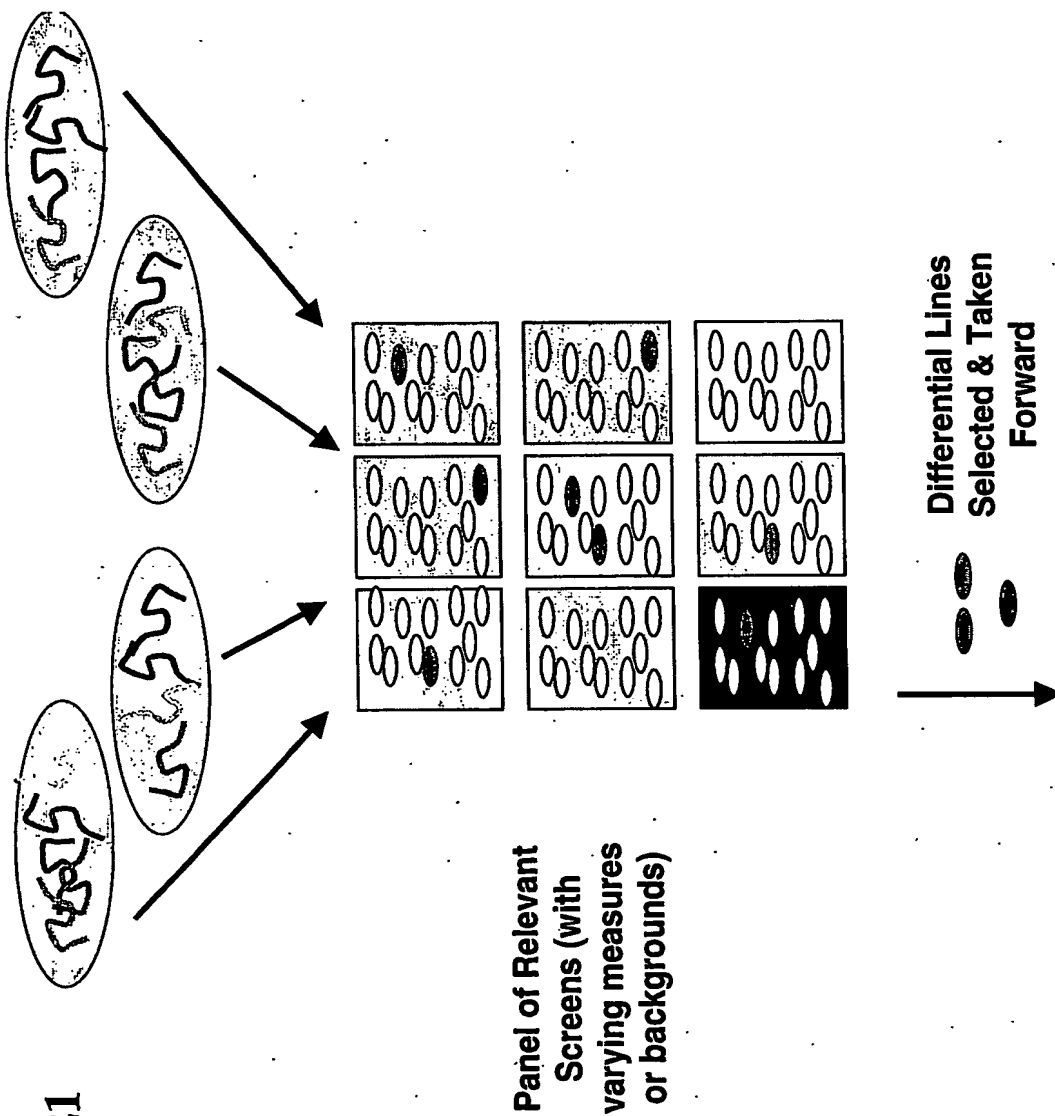
Fig. 20



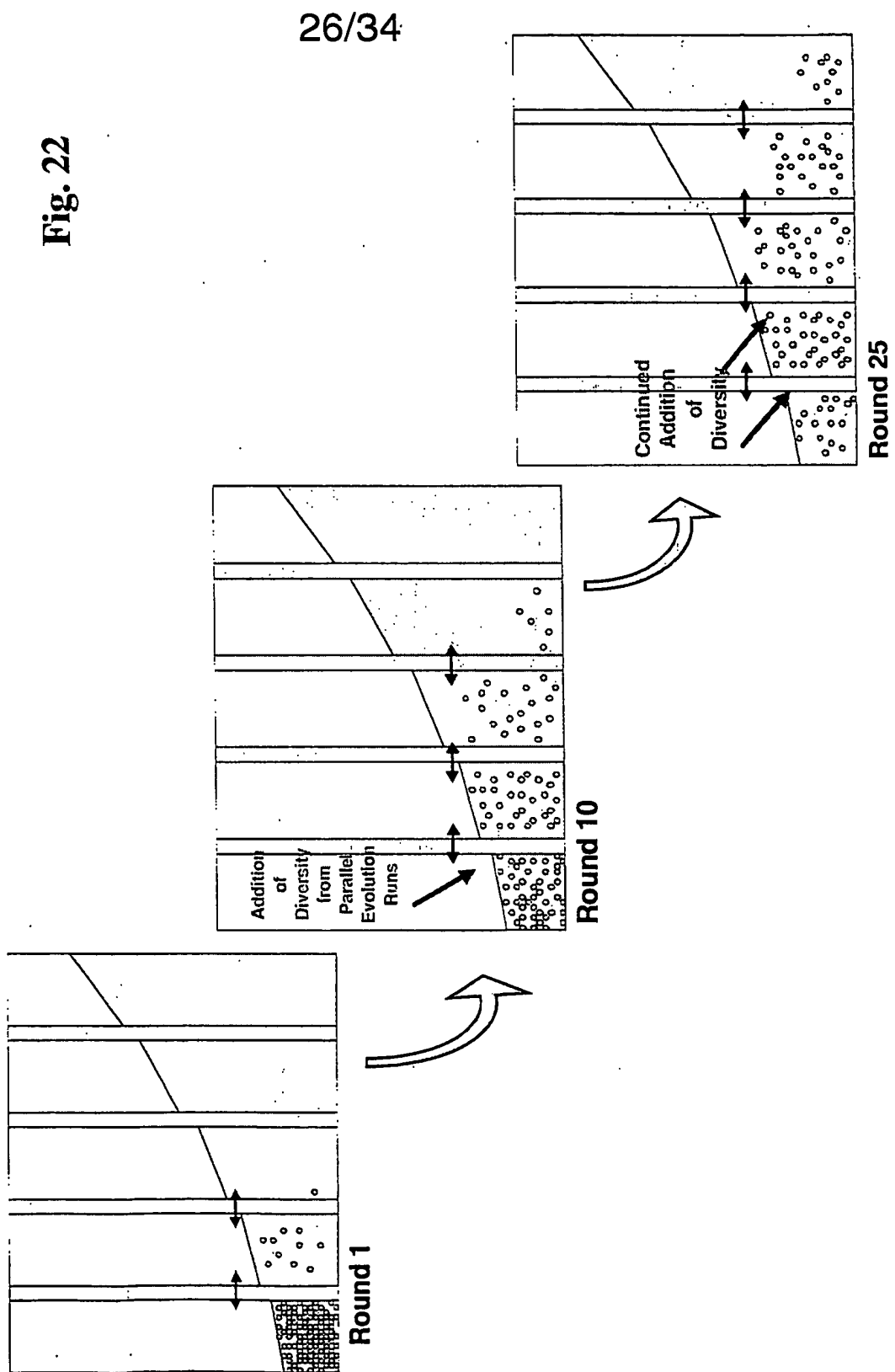
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Screen for Relevant Properties

Fig. 21



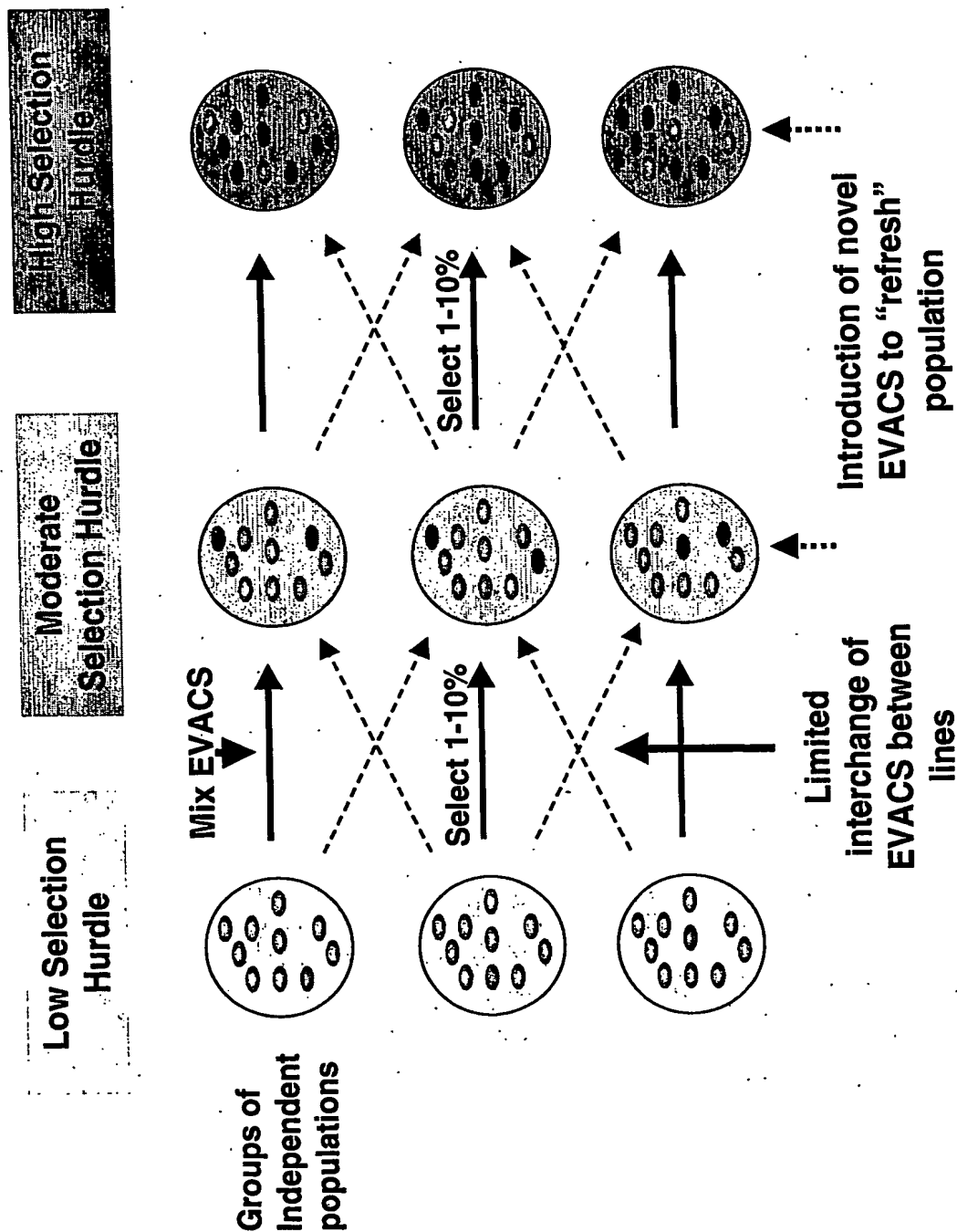
Evolve Through Tiered Screens



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General Screening Strategy

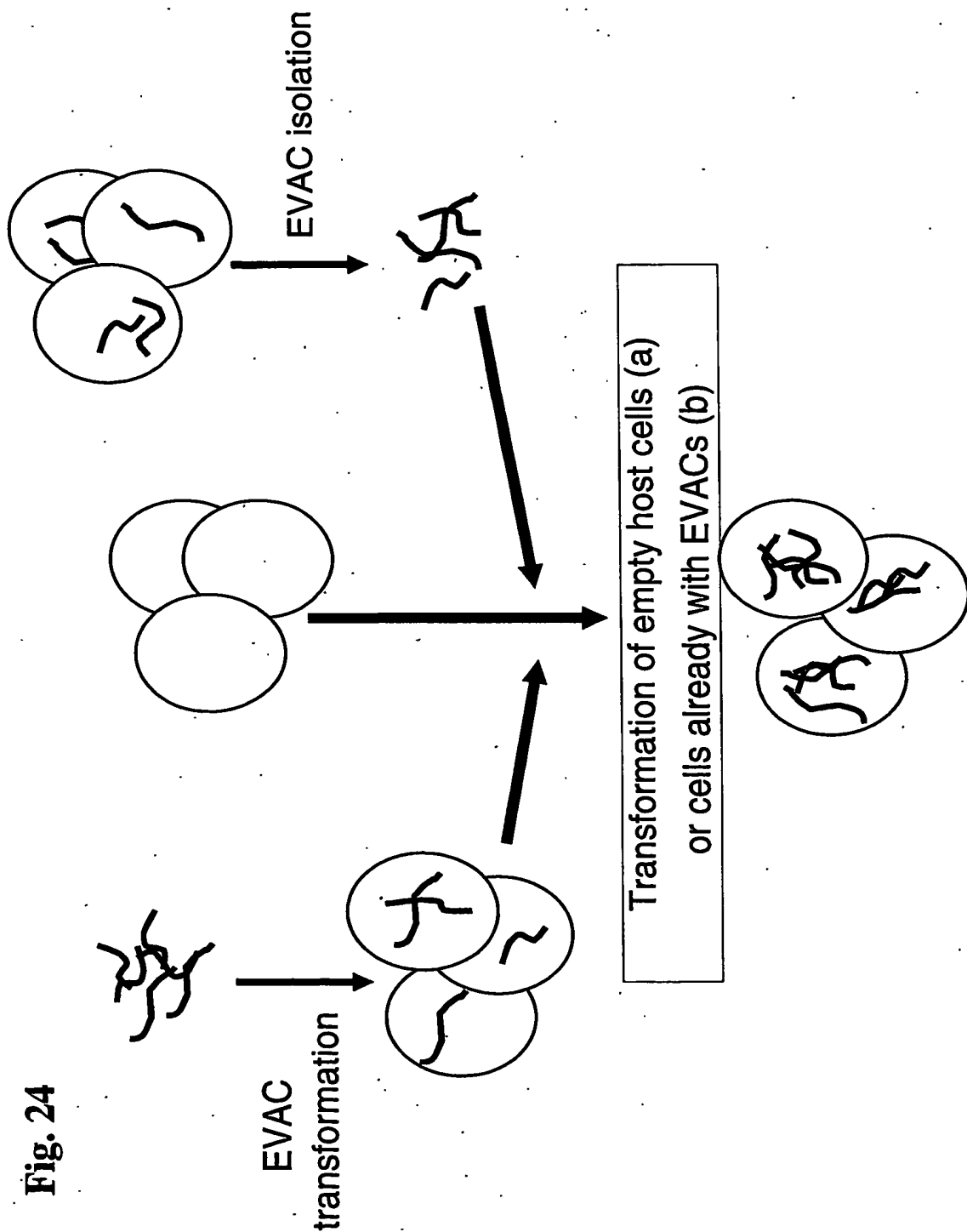
Fig. 23



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Physical EVAC remixing

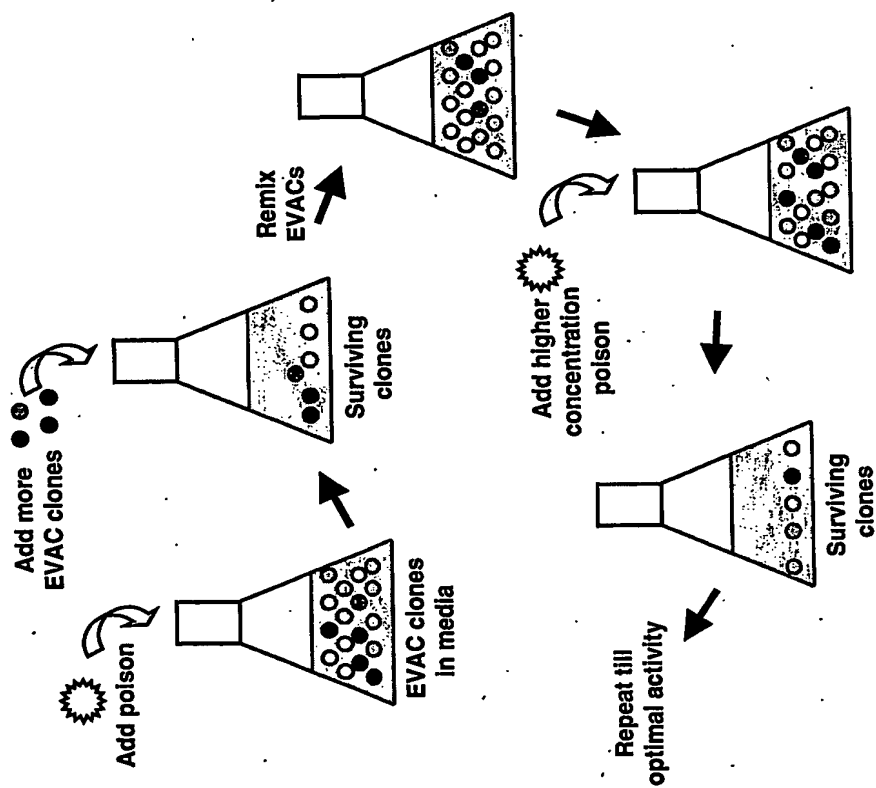
Fig. 24



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Example of Evolution I Selection pressure: cell survival

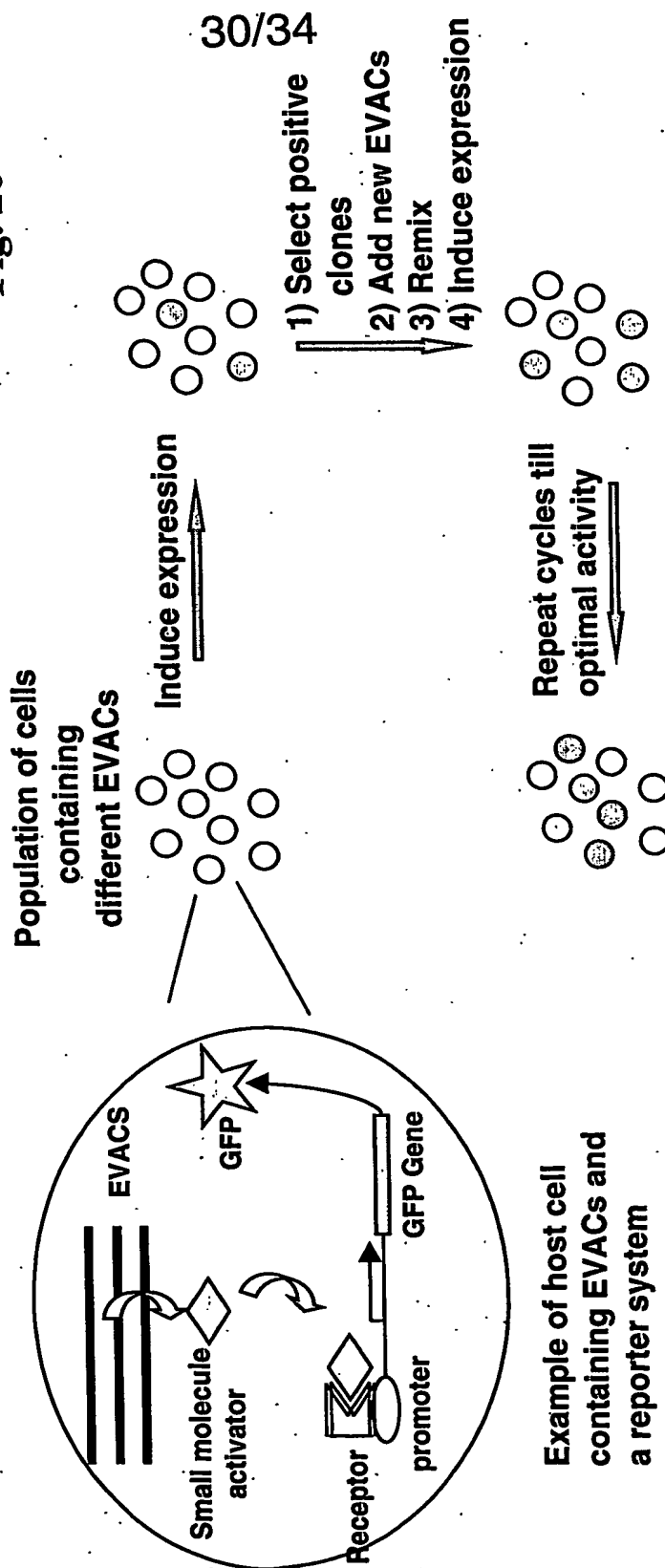
Fig. 25



Example of Evolution II

Selection pressure: activation of a reporter system

Fig. 26



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Example of controllable gene expression

Fig. 27

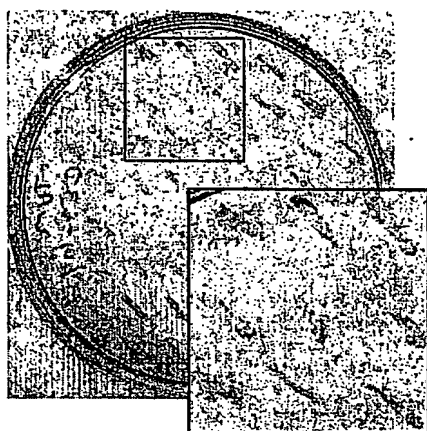


Plate 2: Met Promoter de-repressed

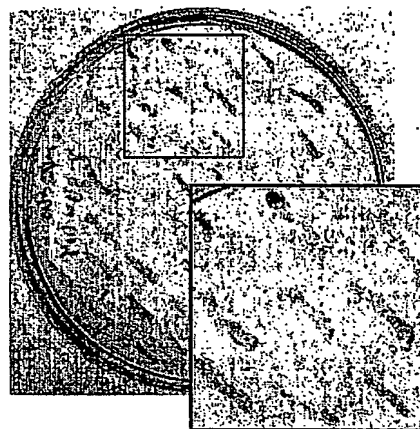


Plate 4: Promoters induced and de-repressed

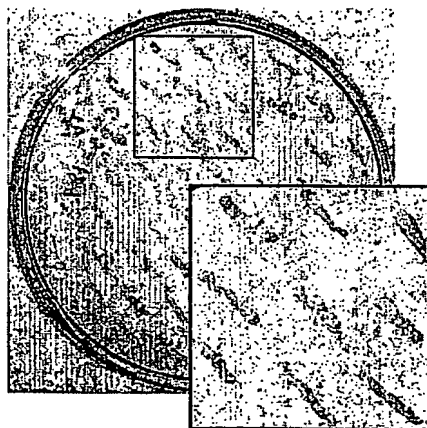


Plate 1: Promoters not induced/repressed

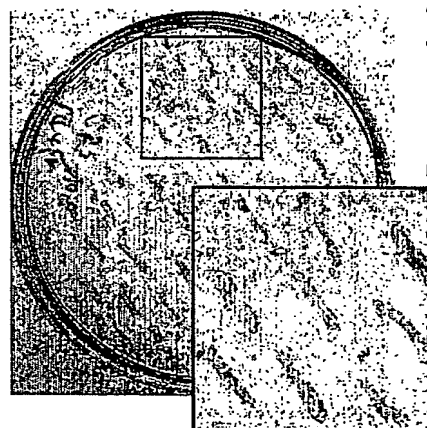
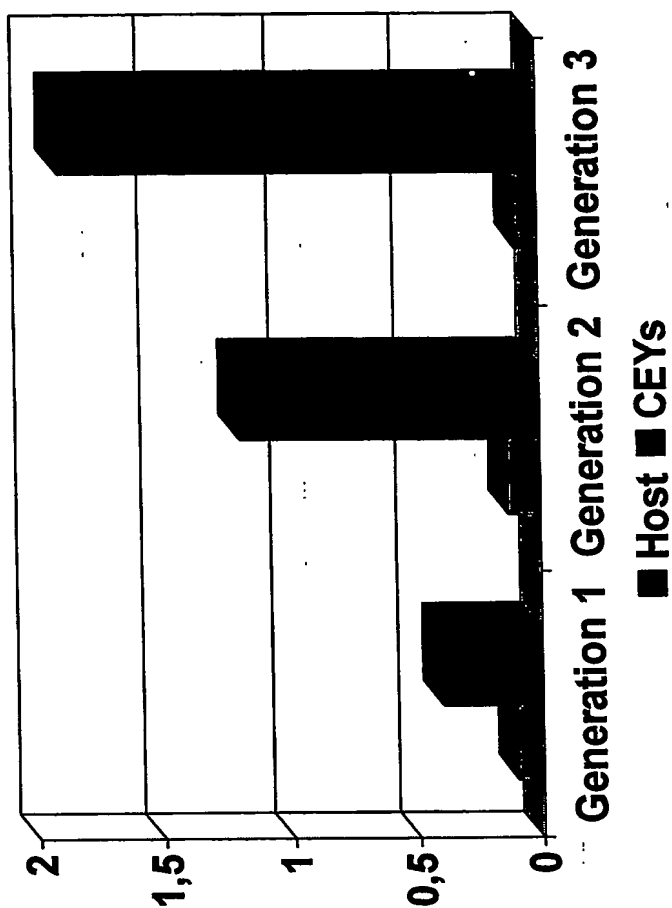


Plate 3: CUP Promoter induced

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**Oxidative Stress Resistance of
Best 1% of cells***

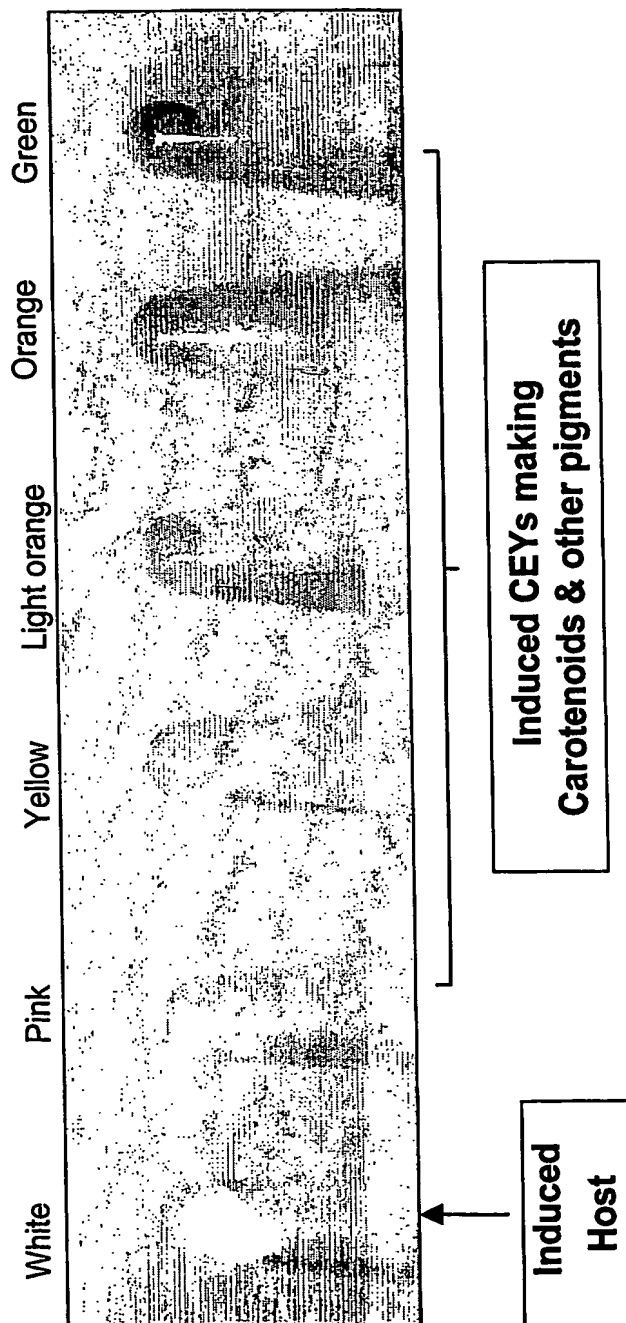


* Methylene Blue Concentration (mM)
where 1% cell population survived
after 2 hours irradiation

Fig. 28

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Fig. 29



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Fig. 30
Carotenoid content (µg carotenoid/g dry weight)

	Cey1	Cey2	Cey3	Cey4	Cey5	Cey8(b)	Cey9(a)	Cey9(b)	Cey11(a2)	Cey11b
absolute	65.39	33.6	1.12	2.91	1.11	50.5	32.50	84	150	134.7
astaxanthin								11,72		6,85
canthaxanthin	3.16	1.95					1.05	20.96	3.3	34.88
echinenone	18.08	6.54		4.55	2.45		11.99	6.09	36.55	18.46
lycopene				9.5				17.80		14.5
neurosporene	1.8	3.21	0.34	6.15		15.57	3.26		13.11	
beta-carotene	32.29	3.69	9.8	21.7	6.35		3.27		84	4.95
427nm-carotenoid, not identified										
405nm-keto-carotenoid, oxidation product			0.11							
440nm-ketocarotenoid, untypically			0.41							
445nm-lycopene				6.21						
beta-cryptoxanthin								5.34		
460nm-carotenoid				12.76				22.05		14.84
beta zeaxanthin									10.5	
zeaxanthin						17.89				
keto carotenoid, not identified						17.26				
465nm-keto-carotenoid, not identified										17.38
460nm keto carotenoid, oxidation product										5.34
445nm-keto-carotenoid, not identified										10.62
phytoene			4.1		1.3		4			